ELECTRONIC/PROTONIC CHARGE CARRIER RATIOS IN SOLVATED BIOMACROMOLECULES

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This is to certify that the

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RATIOS IN SOLVATED BIOMACROMOLECULES

presented by

Michael Robert Powell

has been accepted towards fulfillment
of the requirements for

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Barnett Rosenberg
Major professor

Date Nov. 27, 1969.

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ABSTRACT

ELECTRONIC/PROTONIC CHARGE CARRIER RATIOS IN SOLVATED BIOMACROMOLECULES

By Michael Robert Powell

The change in the ratio of electronic to protonic charge carriers in solvated systems has been investigated for hemoglobin, cytochrome-c, lecithin, melanin, DNA, and collagen. The electrical properties in the solid state of these biomacromolecules follow the operational definition of a semiconductor, i.e., the conductance is given by the equation $\sigma = \sigma_0 \exp(-E/2kT)$, where σ is the conductivity, and E is the activation energy.

These compounds exhibit multilayer adsorption and show a conductivity increase when adsorption occurs. The adsorption process follows Roginsky-Zeldovich kinetics which indicates that the water is penetrating into the crystal; the kinetics of the current increase during the hydration process is rapid (does not follow the Elovich Equation).

The current *vs.* hydration curves show a saturation of the current after 2 to 2½ BET monolayers have been adsorbed. It is at this point that the capacitance of the samples (hemoglobin, cytochrome-c, and lecithin) shows a further rapid increase upon additional hydration.

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The adsorption process can be expressed by either the Bradley or the BET adsorption isotherm. The former suggests the possibility of ordered (polarized) multilayers.

Tracer studies using tritiated water adsorbed on hemoglobin and collagen showed small yields of tritium as compared to the same number of coulombs passed through liquid water.

Solid state electrolysis experiments determining the current efficiency for hydrogen generation allows one to determine the electronic/protonic charge carrier ratios. The change in this ratio, for most materials, is found to be linear with increasing hydration.

Electrolysis of hemoglobin at one hydration state of 28% water showed that protonic conductivity decreases at potentials of less than 50 volts and is constant from 50 to 300 volts. Large deviations from Ohm's law are found at applied potentials of less than 30 volts.

There does not appear to be a sharp onset of protonic conduction at 2 BET monolayers (except for water on DNA and methanol on hemoglobin), but rather this species of carrier seems to be intrinsic in a manner similar to electronic conduction.

ELECTRONIC/PROTONIC CHARGE CARRIER RATIOS IN SOLVATED BIOMACROMOLECULES

By

Michael Robert Powell

A THESIS

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I wish to express my gratitude to Professor Barnett Rosenberg whose patience and encouragment has aided in this effort. I wish also to thank Drs. J. O. Williams, D. W. Boniface, and E. Postow for their many helpful discussions. The financial assistance of the United States Atomic Energy Commission (AT 11-1 -1714) is gratefully acknowledged.

TO MY WIFE AND PARENTS

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I. INTRODUCTION

Organic Semiconductors

Albert Szent-Györgyi (1941), taking up an idea expressed to him by one of his graduate students about the similarity in order between organic and inorganic crystals, proposed that electronic "energy bands" could be found in biological compounds. This similarity was based upon the periodic array found amongst the amino acids in the hydrogen-bonded crystals.

A method of energy transfer and charge transport became possible which did not involve the diffusion of molecules. This was particularly important for energy transfer in solid structures such as the cytochromes of the mitochondria.

The war interrupted the testing of this valuable idea for several years. It was not until later that combinations of proteins and dyes showed that energy migration could take place in these photoactivated systems.

Not until 1949, when Evans and Gergley (1949) published their calculations, was anything known about the possible existance of these electronic energy bands. The calculation

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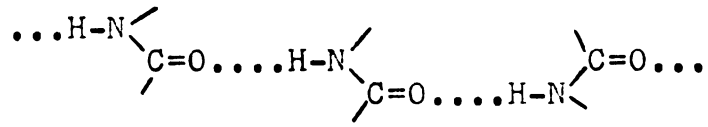

and gave a band gap of 3 e.v. between the highest filled and lowest empty band.

Measurements of the electrical conductivity of violanthrone, iso-violanthrone, and pyranthrone by Akamatu et al.(1950) showed that they were semiconductors with an activation energy E of 3/4 to 1 electron volt, and ovalenen (Akamatu et al., 1951) was found to have E= 1.13 e.v. Bayliss (1948) calculated the spectra of conjugated polyenes using the assumption that the π electrons were "free", as in a metal, and he suggested a role for them in photoconduction.

An extensive study of organic semiconductiong compounds has been carried out in the laboratory of D. D. Eley. Pressed powders were studied by Eley et al.(1953), and they observed that the resistances and activation energies decreased with increasing compression; the presence of water also lowered the activation energy. They did not find a direct correlation between the number of π electrons and the conductivity but did conclude that "linear polyenes are less effective semiconductors than polynuclear systems with a similar number of π electrons"; they associated the conductivity with the π electrons of the aromatic molecules. Using an a.c. method Eley and Parfitt (1958) found that the

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activation energies were intermediate between the pressed (80 kg./cm.^{2}) and unpressed samples of metal-free pthalocyanine and isodibenzanthrone. The values were, pthalocyanine: 1.49 e.v. (a.c.), 2.39 e.v. (d.c., zero compression), and 1.11 e.v. (d.c., 80 kg./cm.^{2}); for isodibenzanthrone: 0.96 e.v. (a.c.), 1.54 e.v.(d.c., zero compression) and 0.74 e.v. (d.c., 80 kg./cm.^{2}). The radical α - α diphenyl β - picryl hydrazyl gave an activation energy of 0.26 e.v. (a.c.) and 1.49 e.v. (d.c., light compression). To account for the low activation energy of the radical, a band picture was utilized where the odd electron was already in the conduction band so that E became the energy needed for intermolecular transfer.

The studies of hemoglobin by Cardew and Eley (1959) showed an activation energy of 2.75 e.v. for dry hemoglobin and 2.97 e.v. for dry globin(some dependence on pressure was noted for both compounds). The activation energy difference between compressed powders and single crystals (a.c.) amounted to only 0.2 e.v. It was reasoned that the charge carriers were electronic in the dry state because (i) steady state values of the current were reached within one minute, (ii) the effect of decomposition was to give a lower conductivity -- higher conductivities would be expected if the carriers were ionic, and (iii) the σ_0 values were 10^3 to 10^5 times smaller than polyamides -- suspected protonic conductors. They agreed that adsorbed water would induce a protonic conductivity in the solid proteins.

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Studies of water adsorption on hemoglobin by Cardew and Eley (1958) gave values for the BET monolayer which differed for the freeze-dried and alcohol-denatured hemoglobin. For native material, they calculated v_m, the BET monolayer, as 5.76 g/100 g. protein (30° C.) and 5.72 g/100g. (40° C,), and for the denatured material they found 5.52 g./100g. (30° C,) and 5.48 g./100g. (40°C,). These values supported the idea that the water was adsorbed by the polar side chains on the surface of the molecule; about 73% of the side chains were involved in the adsorption process.

Water was found by Eley and Spivey (1960 a) to greatly increase the conductivity of hemoglobin; because of an artifact, this increase was not as large as is generally seen. It was postulated that water donated electrons into the conduction band of the hemoglobin. Eley and Spivey (1960 b) found that alcohol denaturation changed the value of E from 2.36 e.v. for native hemoglobin to 2.88 e.v. for denatured material; there was only a small change in the value of σ_0.

The possibility of electronic carriers in slightly hydrated hemoglobin (7.5% water) being dominant over protonic carriers was tested by Rosenberg(1962 a) in an experiment where a current decrease with time was checked for. The absence of this decrease indicated that the water was not being electrolysed, and therefore charge transport by the water was negligible.

A study by Rosenberg (1962 b) of water and hemoglobin

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indicated that the water served only to reduce the activation energy for charge carrier generation, and it did not affect the value of σ_o .

Rosenberg (1964 a) made an attempt to measure the amount of protonic charge carriers utilizing a tracer technique with tritiated water. At a hydration state of about 30%, he found 44% protonic conduction.

On the basis of the few measurements which had been made, Rosenberg (1964 b) suggested that the charge carriers were electronic in the range where the current increases exponentially with hydration. In the region where saturation of current occurs, the carriers would be mixed ionic and electronic, and in the range where the amount of adsorbed water is high, the carriers would be primarily protonic.

Eley and Leslie (1964) found that the electrical conductivity for denatured hemoglobin was greater than native hemoglobin for the same hydration state. They also found that there was no saturation point in the current at the first BET monolayer as they had earlier found; this finding was in agreement with the finding of Rosenberg (1962 b). They proposed that the energy gap E_d decreases with hydration with the form

$$E_d = E_d^{\,o} - a\, n_d^{\,1/3} \qquad (1)$$

where n_d is the amount adsorbed, and the exponent $1/3$ is derived on the basis that the interaction energy between impurity molecules is inversly proportional to the distance

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between them. By plotting log conductivity vs. (water adsorbed)$^{1/3}$, they obtained a straight line which showed current saturation at high regain.

The adsorption of water on hemoglobin was found to follow Roginsky-Zeldovich kinetics (Eley and Leslie, 1966). This implies an increase of activation energy for adsorption with increasing value of regain. They explained this by an electron injection mechanism where the water donates electrons to the protein. Thus the adsorption follows the process:

$$H_2O + \text{Protein} \rightleftharpoons (H_2O...\text{Protein})_{VW} \longrightarrow (H_2O^+ \rightarrow \text{Protein}^-)_{CT}$$

and the rate-limiting step is the transition from the Van der Waals state (VW) to the charge transfer state (CT). They modified their original equation (Equation 1) such that the exponent of n_d was now unity (1).

Maričić, Pifat and Pravdić (1964) attemped an electrolysis on hemoglobin at various hydration levels. Their proceedure was to measure the increase of volume of the electrolysis cell during the release of hydrogen (this was accomplished by noting the movement of a slug of mercury in a capillary). Their data indicated protonic conductivity only at 18% hydration or above, but the scatter in their data on the amount of protonic conductivity (20% to 94%) made interpretation very difficult indeed.

The hydration of hemoglobin produces changes in the dielectric constant. Brausse et al. (1968) found that the dielectric constant slowly increased with hydration, and at

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about 16% regain (2½ BET monolayers) it increased rapidly and became constant at about 35% regain. The dispersion was attributed to Maxwell-Wagner polarization. A large change in the loss tangent at 15 % hydration was noted by Maričić and Pifat (1966). Measurements of the dielectric constant of hemoglobin with adsorbed water, ethanol, methanol, and ammonia by Postow (1968) showed that the increase in conductivity could be explained by the increase in the dielectric constant according to the equation of Rosenberg (1962 b).

Similar effects of capacitance increase with hydration have been noted by Rosen (1963) on bovine serum albumin. He noted a large change in the capacitance at about 20% regain. Takashima and Schwan (1965) found similar effects by water on the capacitance of ovalbumin; the large capacitance change was seen at about 12% for this protein.

The porphyrin prosthetic group of hemoglobin, ferriheme, was found by Cardew and Eley (1959) to have an activation energy of 1.74 e.v., considerably below the value of 2.74 e.v. found by them for hemoglobin. A change of the central metal atom effected only small changes in the activation energy (Eley and Spivey, 1962).

The investigation of DNA was undertaken more from the theoretical point of view of solid state biophysics than for any definite implication in living systems, although there may be some relation between conduction and carcinogenesis or radiation damage. Löwdin has also produced

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a theory to explain mutations based upon the movement of protons from one base to another; these movements are generally of short distance, however.

Studies by Eley and Spivey (1962) on thymus DNA gave an E= 2.42 e.v. for vacuum dried samples; the energy gap for yeast RNA was 2.42 e.v. In that polarization effects were small, they predicted that dry nucleic acids were electronic semiconductors.

Eley and Leslie (1963) studied the activation energies of nucleic acid componentsand found the following values for E :(a) nucleoside (base + ribose) 4.5 - 5.2 e.v., (b) nucleotide (base + ribose + phosphate) 2.0 - 2.2 e.v. This last value is comparable to DNA and RNA, each of which have an activation energy of about 2.4 e.v. They suggested that the conduction state would correspond to a $\pi - \pi^*$ excitation (or $n-\pi^*$), using a band theory approach for DNA; for the nucleoside, a $\pi - \pi^{**}$ excitation would be necessary.

Solid-state electrolysis experiments were made on Na-DNA by Maričić and Pifat (1966) with the finding that hydrations of 37% and higher gave very large values for the amount of protonic conductivity. Unfortunately, the poor degree of precision in the experimental values was not amenable to a good interpretation of their results. It was their contention, however, that at hydrations of less than 37%, the amount of protonic conductivity was small or even non-existant.

Burnel et al.(1969)reëxamined the semiconduction parameters of the nucleic acids and found an E= 2.36 e.v. and a $\log \sigma_o$ of 2.35 for DNA and RNA. They also found that Poly-A and Poly-C had dry-state activation energies of 2.2 and 2.4 e.v., respectively. The similarity between the electrical properties of these two compounds and DNA has an important consequence. Both Poly-A and Poly-C retain their crystalline character even when desicated as opposed to DNA which becomes disordered upon drying. Thus the similarity of semiconductivity properties of dried material suggests that base-stacking is of a small consequence for electronic conduction.

It was postulated,then, that the activation energy for charge carrier generation represented the energy needed to remove an electron from the bases and place it on the phosphate of the backbone; the stacked bases would then be hole conductors and the backbone would be an electronic conductor. The excited state was then an optically inactive, charge-transfer state.

J. Ladik (1968) believes that the high frequency activation energy (0.2 e.v.) found by O'Konski et al.(1964) represents the energy gap for a single DNA molecule, and the higher d.c. values of E = 2.34 e.v. represent the intermolecular energy barrier. Ladick calculates a band gap of about 3.7 e.v. ,and he concludes that DNA is an inhomogeneous impurity semiconductor.

A-form DNA contains large amounts of water (Falk, et

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al., 1663 a, b)and is stable only at values of p/p_o greater than 0.7. Calculations of Sklenar, Ladik,and Biczo (1967) of water and Poly G-C showed only minor modifications of the band structure with respect to systems not containing water.

Making use of proton-injecting electrodes (hydrogen-saturated palladium), Thomas et al. (1969) found large current increases with the pyrimidine base isocytosine. The difference between the current in the presence and the absence of hydrogen was greater than 10^{10}.

Studies of the d.c. conductivity of Na-DNA by O'Konski, Moser and Shirai (1964) showed regions of non-ohmic behavior in a hydrated sample ($p/p_o = 0.31$) with potentials of from 0 to 200 v. $cm.^{-1}$ at 25° C.; at larger potentials the current was ohmic. From various observations (the effect of O_2, photoconduction, and space-charge effects), they concluded that dry DNA was an electronic conductor, and that hydrated samples likely possessed a large complement of ionic conduction.

Eley and Spivey (1960) measured the semiconduction activation parameters for dry cytochrome-c and found an $E = 2.60$ e.v. and a $\log \sigma_o = 4.8$; these values being slightly smaller than those which they found for hemoglobin (cf. $E = 2.66$ e.v. and $\log \sigma_o = 5.0$). In a manner similar to hemoglobin, cytochrome-c displayed ohmic behavior up to field strengths of 2,000 v. $cm.^{-1}$ at 115° C. in the dry state.

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Cytochrome-c is of interest to the biologist by virtue of its presence in the oxidative phosphorylation chain. Furthermore, this material appears to be bound to the cristae of the mitochondria making electron transfer by diffusion of the cytochrome to the substrate a rather improbable event.

Two possible mechanisms exist for this electron transfer, the first being semiconduction and the second being quantum-mechanical tunnelling, although in cases where "tunnelling" is the rate limiting step in semiconduction, there is little difference between the two. This latter case might be seen in inter-molecular transfer of charge. At present, there remains a difference of opinion amongst workers in the field as to which is the correct method (if there is indeed a difference), and no irrefragable evidence exists for either side. The present investigation was not undertake to resolve the controversy, but rather to show that, at high degrees of hydration and consequently lower resistances, the component of electronic conductivity was large and could conceivably be used to explain the transfer of electrons. In short, it lends support to, but not proof of, electronic semiconduction in cellular organelles.

A large number of enzymes are present in living organisms which contain transition metal ions. Cytochrome-c, an iron-containing molecule, is one example. A rewiew of this topic has been prepared by R. J. P. Williams (1968).

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resistance of cytochrome-c was too large to adequately account for the observed resipiration rate in the sea urchin egg. They used, however, the dry state activation energy, and from this calculation, they found a discrepancy of 10^{16} between theory and experiment. Using the hydrated cytochrome-c resistances, Rosenberg and Postow (1969) arrived at the conclusion that semiconduction was indeed a possible mechanism to explain respiration in the sea urchin egg.

At present, there is some evidence that the movement of electrons may proceed by a fairly long range tunnelling process (about 60 Å). DeVault, Parkes, and Chance (1967) showed that the oxidation of cytochrome-c in the purple sulphur bacterium could occur at liquid helium temperatures with a time constant of 2.3 msec. and no indication of an activation energy at temperatures of less than 120° K.

Measurements of conductivity and dielectric properties by Taylor (1960) lead him to the conclusion that conductivity was mediated either by anions or by protons (in the dry state) in cytochrome-c.

Electrical Conductivity and Hydration

The history of the conductivity increase in hydrated biomacromolecules is rather extensive, and it can be traced back to the investigations of Evershed (1914) who studied the effect of moisture on materials which were used to insulate electrical wires, such as cotton. He came to the conclusion that practically all of the current increase was

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effected by water condensation on the internal and external surfaces of the fiber.

Murphy and Walker (1928) studied the d.c. conductivity increase of cotton, silk, and wool with adsorbed water and found that the resistance for cotton was given by

$$\log R = -9.3 \log M + B \qquad (2)$$

and for silk by

$$\log R = -16.0 \log M + C \qquad (3)$$

and for wool

$$\log R = -16.4 \log M + D \qquad (4)$$

It was conjectured that the water flowed in water channels, and the fiber resistance depended upon the number of restrictions in these channels; the number of restrictions was reduced with the increase of adsorbed water.

Murphy (1929 a) studied the a.c. conductivity of cotton and silk and found that the parallel conductance increased with frequency, but it became frequency independent at high humidities. He found that the a.c. conductivity could be represented by

$$G = G_o f^n \qquad (5)$$

where G_o and n are constants, and f is the frequency; n was found to be an approximately linear function of the humidity. In a later paper, Murphy (1929 b) discussed the

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Evershed effect, i.e., the decrease of resistance of hydrated materials with increasing applied potential, with the conclusion that it was caused by a combination of a back-emf at low applied potentials and the Wein effect at higher potentials.

Marsh and Earp (1933), working with single wool fibers, found a decrease of resistance with increasing hydration, but the currents were found to be ohmic with no evidence of polarization. It was their opinion that the current was carried in water filled pores in the fiber.

Baxter (1934), however, concluded that the water in the pores could not be of the same form as water in the liquid state in that the activation energy for conduction was different in water than in wool. He obtained the following equation for the conductance:

$$\sigma = A \exp(-E/kT) \qquad (6)$$

where σ is the conductivity, and A is a constant. He proposed that conduction was the result of electron tunnelling between the adsorbed water molecules. The resistance would then vary exponentially with the water-water spacing in that the probability of electron tunnelling from one water molecule to another is proportional to r, the width of the barrier, and this probability is given by

$$P = B \exp(hr) \qquad (7)$$

where B and h are constants. If one assumes a uniform

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distribution of water, then r is proportion to $m^{-1/3}$, where m is the amount of adsorbed water. Thus the resistance could be given by the equation

$$R = C \exp (k\, m^{-1/3}) \qquad (8)$$

where C and k are constants.

Fuoss proposed an ionic conduction mechanism based upon the idea that plasticizing agents facilitate ion migration. This idea was tested by King and Medley (1949 a) with the conclusion that substances such as water and formic acid not only lower the diffusion resistance of the ions in the polymer matrix, but that increased dissociation of the ions also occurs. They electrolysed a keratin-water sample and found that,at 18% adsorbed water, the yield of hydrogen was 92% of the theoretical amount. From this, King and Medley (1949 b) concluded that electrolysis was taking place as was predicted by the ionic theory, albeit, the amount of oxygen found was only 18% of the theoretical amount.

E. J. Murphy (1960) used a variation of the theory proposed earlier concerning the completion of water channels. In the revised picture, the water was adsorbed on specific sites, and these water adsorption sites were proposed to lie between "ion-generating sites". These latter were places where the dissociation energy ,or the ionization energy, for the formation of H^+ was less than for the formation of H_3O^+ and OH^- from water. The ion-generation

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sites consisted of protons which had exchanged for metal ions in an ion-exchange process. The anion to which the proton was bound was a part of the macromolecule (cellulose in the specific case treated by Murphy).

The water molecules were envisioned to connect the easily ionized protons and thus provide a conduction pathway. If the amount of water adsorbed at saturation is α_o, and the amount of water present at any given hydration state is α, then the probability of any site being occupied is (α / α_o). If there are n sites for water adsorption between any two ion-generating sites, then the probability that all of the sites will be filled is given by $(\alpha / \alpha_o)^n$.

The conductivity is then calculated empirically from this probability. The conductivity at saturation is given by σ_s. Thus, for any state less than saturation, the conductivity is given by

$$\sigma = \sigma_s (\alpha / \alpha_o)^n. \qquad (9)$$

The theory is applicable only to protonic conductors, in so far as the idea of ion-generating sites is utilized. However, in the case of cellulose, to which Murphy directed his theory, the primary conductors were found by him to indeed be protons (Murphy 1963, 1965).

Several studies by Eley have led him to the conclusion that the adsorbed water increases the conductivity of proteins by injecting either electrons or holes into the

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conduction bands of the material. As discussed earlier, the energy gap is decreased according to Equation 1. This general equation was found by Pearson and Bardeen (1959) for boron doped silicon. If the water is distributed over the surface of the spherical molecules, the exponent in Equation 1 would be $\frac{1}{2}$, and the equation of the energy gap would then be

$$E_D = E_{D_o} - \alpha n_D^{\frac{1}{2}} \tag{10}$$

According to Eley, the total equation for the conductivity then becomes

$$\sigma = e\mu(2n_D)^{\frac{1}{2}} \left[\frac{(2\pi mkT)^{3/4}}{h^{3/2}}\right] \exp -\left[\frac{(E_{D_0} - \alpha n_D^{x})}{2kT}\right] \tag{11}$$

or

$$\log \sigma = \text{Constant} + \log n_D^{\frac{1}{2}} + \beta n_D^{x} \tag{12}$$

where x is a function of the distribution of the physically adsorbed water on the molecule and of the forces between the adsorbate molecules; in the case of inorganic compounds, x is equal to 1/3.

Another theory proposed by Rosenberg (1962 b), based upon the change of the dielectric constant of the conducting medium will be discussed in the next chapter.

Adsorption Isotherms

The adsorption of gases on solids is a large problem in itself, but for this study, it was used only as a tool

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and not as an end in itself. Much discussion in the past has centered around the change in the carge carriers as the amount of adsorbate increases; this change was often thought to occur in the hydration range of from two to three Brunauer-Emmett-Teller (BET) monolayers. This prediction was investigated as a part of this study.

Brunauer (1943) has distinguished several different types of adsorption isotherms, and these are shown in Figure 1. The first is Langmuir or monolayer adsorption and is known as Type I. It follows the equation

$$v = (kbp)/(1 + bp) \tag{13}$$

where v is the amount of gas adsorbed , p is the pressure, and k and b are constants specific to the system under investigation. An example of this type is the adsorption of O_2 or N_2 on carbon or silica gel at low temperatures.

Type II isotherms are commonly referred to as BET or multilayer adsorption. In this case, the adsorbate adsorbs first on the primary sites, and this is later followed by the buildup of molecule upon molecule until several layers result. These isotherms are a very common case of physical adsorption, and follow the equation

$$v = \frac{cpv_m}{(p_0-p)\left[1 + (c-1)(p/p_o)\right]} \tag{14}$$

where v is the amount adsorbed, p is the pressure, p_o is the saturation vapor pressure, v_m is the amount of adsorption for the first monolayer, and c is a constant

Type III
Type II
Type I

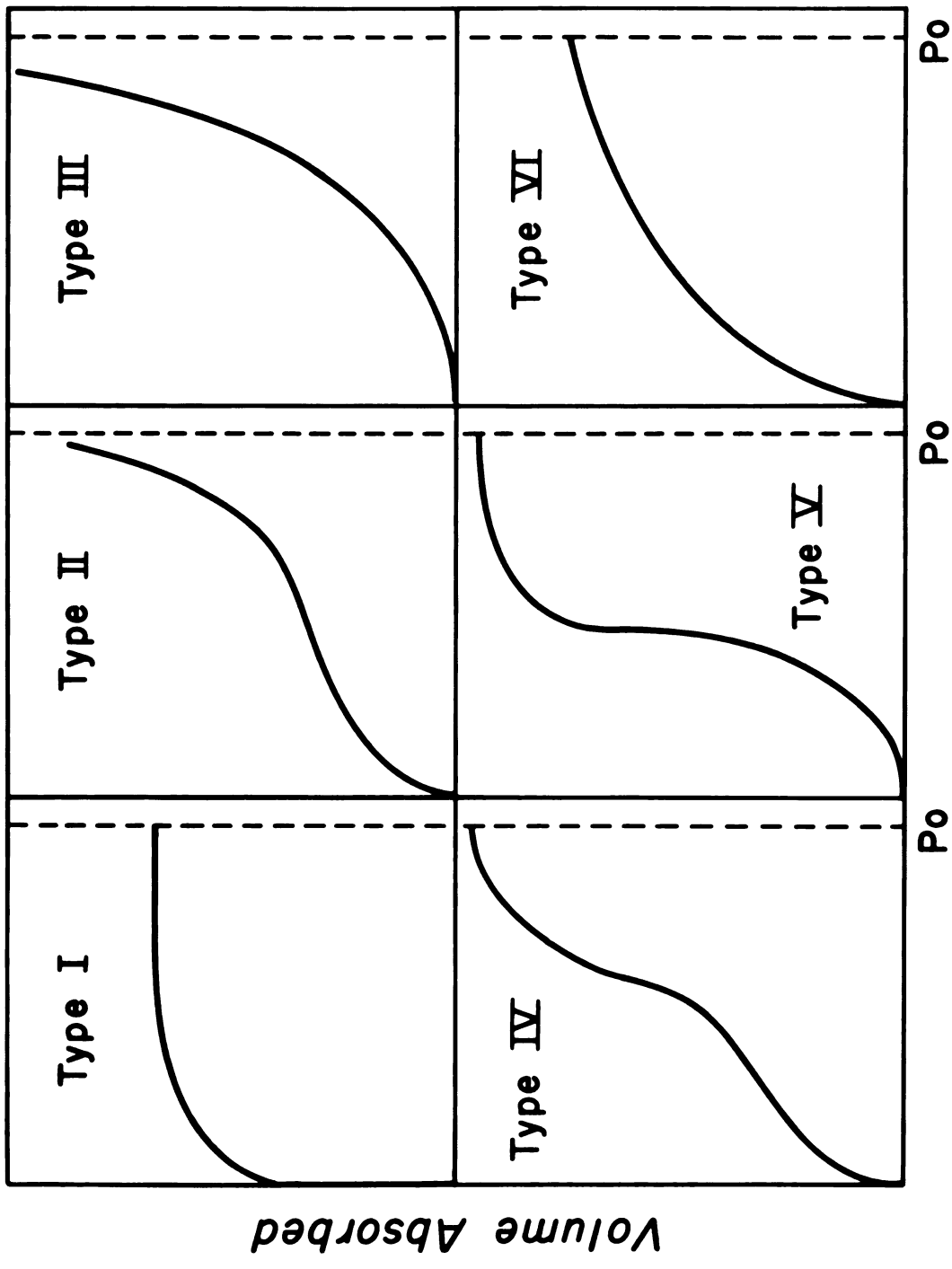

Figure 1. The six adsorption isotherms for physical and chemical adsorption.

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$$c = \exp\left[(E_1 - L)/ RT\right] \qquad (15)$$

where E_1 is the heat of adsorption of the first monolayer, and L is the heat of vaporization.

Type III are rare and are the result of molecular forces which are stronger between the adsorbate molecules than between the adsorbate and the adsorbent. That is to say, $E_1 < L$. An example of such a system is bromine on silica gel at 79° C. or water on graphite.

Capillary condensation is thought to be responsible for Type IV isotherms. In this type of system, a significant amount of vapor is believed to condense in small pores. When these pores fill, saturation is reached. In Type IV isotherms, E_1 is greater than L.

Similar to Type IV, the Type V isotherm is the result of capillary condensation. The distinction between the two is the result of the difference between the binding force between adsorbent and adsorbate such that for Type V, $E_1 < L$.

Type VI is characteristic of chemisorption processes, that is, those instances where covalent or ionic bonds are formed as contrasted with physical adsorption where only weak forces are involved. In chemisorption, the heat of adsorption is large as compared with physical adsorption.

By an appropriate arrangement of the BET equation, it is possible to graph the experimental results and obtain

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v_m , the monolayer coverage.

Adsorption isotherms for very polar adsorbates may also be calculated by means of the equation of Bradley (1936). It is a modification of a theory originally proposed by DeBoer and Zwicker (1929) in which the more polar surface causes an induced dipole to be formed in the adsorbed layer. This layer polarizes the layer above it et cetera.

The equation, if lacking certain theoretical verisimilitude, is a valuable empirical tool for describing the experimental results over values of p/p_o from 0.05 to 0.9. This is in contrast to the BET theory which is linear only from p/p_o of 0.05 to 0.5 in general.

The range of the Bradley equation, more than specific molecular or physical information about the adsorption process, was the reason for its use. This was true for the methanol-hemoglobin system where mechanical problems with the balance made it impossible to solvate the sample at a p/p_o of greater than 0.75. For all other studies, the BET equation, with its value of v_m, proved to be the most valuable.

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II. THEORY

Electrical Conductivity

Rosenberg (1962 b) has proposed a theory of the electrical conductivity increase upon hydration using the premise that the effective dielectric constant will be increased. This will lead to a reduction in the energy needed to separate the positive and negative charges which account for the current.

Many biomacromolecules follow the operational definition of a semiconductor, that is, they follow the equation

$$\sigma = \sigma_o \exp(-E/2kT) \qquad (16)$$

Their conductivity increases with increasing temperature. This is contrasted with metallic conductors which show a resistance increase with increasing temperature. The number 2 in the denominator of Equation 16 is customary in the field of organic semiconductors and is the legacy of band theory. While there is no proof that energy bands exist in these organic compounds as they do in inorganic semiconductors, the formalism of band theory is still followed, and the "2" persists.

Of interest is the fact that organic semiconductors change their conductivity upon hydration; this change can

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be represented by the equation

$$\sigma_m = \sigma_{Dry} \exp(\alpha m) \qquad (17)$$

where m is the amount of water adsorbed, and α is a constant. Rosenberg (1962 b) has shown that σ_o does not change upon hydration, but instead it is E which is a function of hydration. It is therefore possible to combine Equations 16 and 17 and obtain

$$\sigma(T,m) = \sigma_o \exp\left[(-E_D / 2kT) + \alpha m\right] \qquad (18)$$

where E_D is the dry state activation energy. The activation energy E is then

$$E = E_D - 2kT\alpha m \qquad (19)$$

The question is how to calculate E, the activation energy of the solvated system, using a phenomenological approach, without recourse to αm, so that one accrues a better physical picture of the processes involved.

If one desires to move free changes in the presence of an applied field, the first prerequisite is the generation of the charges themselves. This process can be thought of as simply removing an electron from the molecule and placing at a point where the coulombic attraction is now less than kT. The energy for this process would correspond to the ionization potential of the molecule. If the charge is placed on another molecule, extra energy, the "electron affinity", is returned. In addition, the lattice

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will relax in such a fashion that the dipoles will orient themselves to further lower the energy of the system; this energy is termed the "polarization energy". The polarization energy is multiplied by two in that both the negative and positive charges are included. The energy for the total process in the dry state would be

$$E_D = I_g - A_g - 2P \qquad (20)$$

where the ionization potential, I_g, and the electron affinity, A_g, are taken as the gas phase values. This polarization energy is comparable to the orientation of solvent molecules around a dissolved ionic compound.

The amount of the polarization energy for spherical symmetry is given by

$$P = (e^2/ 2R) (1 - 1/\kappa) \qquad (21)$$

where e is the electron charge, R is the radius of polarization, and κ is the dielectric constant of the medium. The actual value of κ can not be calculated without a knowledge of the positions of all of the dipoles involved, so the bulk dielectric constant is used as a first approximation. If Equations 20 and 21 are combined, the following equation results:

$$E_D = I_g - A_g - (e^2/R) (1 - 1/\kappa) \qquad (22)$$

For the hydrated systems, the value of κ will change; this new value is represented by κ'. The gas phase

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ionization energy and electron affinity are not functions of hydration. The new energy for hydrated systems is then

$$E = I_g - A_g - (e^2/R)\ (1 - 1/\kappa') \qquad (23)$$

Combining Equations 22 and 23 yields

$$E = E_D - (e^2/R)\left[(1/\kappa) - (1/\kappa')\right] \qquad (24)$$

and from Equations 19 and 24 we have

$$2kT \propto m = (e^2/R)\left[(1/\kappa) - (1/\kappa')\right] \qquad (25)$$

Substituting (25) into (19) gives the final result

$$\sigma(T,\kappa') = \sigma_o \exp(-E_D/2kT).\ \exp\left\{(e^2/2kTR)\left[(1/\kappa) - (1/\kappa')\right]\right\} \qquad (26)$$

Adsorption Isotherms

These were used only to determine the monolayer coverage, but a few words, at least, should be said about them. Specifically, the two systems used to analyse the data were the BET and the Bradley isotherms.

In the BET theory, it is assumed that a specific heat of adsorption exists for the first monolayer; all subsequent layers will yield a heat equal to the heat of vaporization of the compound being adsorbed.

The BET model assumes that there exist particular adsorption sites, and, at any one time, S_o of these will be free, S_1 will have one molecule adsorbed, S_2 will have two molecules adsorbed and so on. When the system is in a state of equilibrium, a certain probability of coverage will exist on each site. For the first monolayer, the

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ratio of covered to uncovered sites will be

$$S_o/S_1 = (b_1/a_1 p) \exp(-E_1/RT) \qquad (27)$$

where a_1 is a constant from kinetic theory, p is the vapor pressure, b_1 is a constant (a function of the frequency of molecular oscillation), E_1 is the heat of adsorption of the first monolayer, and R and T are the gas law constant and the absolute temperature, respectively.

For each subsequent layer, the adsorption is

$$S_{i-1}/S_i = (b_i/a_i p) \exp(-E_i/RT) \qquad (28)$$

If x represents the total amount of vapor adsorbed for any given pressure p, and x_m is the monolayer coverage, it is possible to show that

$$x/x_m = \frac{Cy}{(1 - y)(1 - y + Cy)} \qquad (29)$$

where C is given by

$$C = \exp\left[(E_1 - L)/RT\right] \qquad (30)$$

in which L is the heat of vaporization, and y is p/p_o. This yields the familiar expression for the BET isotherm

$$\frac{p}{x(p_o - p)} = \frac{1}{x_m C} + \left[\frac{C - 1}{x_m C}\right]\frac{p}{p_o} . \qquad (31)$$

Thus a plot of $p/x(p_o - p)$ *vs.* p/p_o yields a straight line whose slope is $(C - 1)/x_m C$ and whose intercept is $1/x_m C$. From this the monolayer coverage x_m may be determined in that there are two equations for the two unknows (x_m and C).

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The isotherm developed by Bradley has its main use in cases where the adsorbate possesses a large permanent dipole moment. This is true for water and methanol (1.85 and 1.70 Debye units, respectively), although gases such as nitrogen and argon (not tested here) are not believed to follow this theory so closely. This isotherm was used here to predict the solvation state of the methanol-hemoglobin system.

The derivation of the equation is not given here in that the theory on which it was developed was not correct for the general case (cf. Brunauer et al., 1938). Thus the Bradley equation is mainly empirical. In its general form, the isotherm is described by the equation

$$T \log_{10} (p_o/p) = K_1 K_3^{A} \tag{32}$$

where T, p, and p_o have their usual significance in adsorption theory, and K_1 and K_3^{A} are defined by

$$K_1 = \frac{N\mu^2}{2.3R}\left[\frac{1}{2a} - \frac{4}{a_1^3}\right]\left[\frac{(3 - k^2)}{(1 - 2k^2)^2}\right]\left[\frac{(1 - k^2)^6}{k^2}\right] \tag{33}$$

and

$$K_2 = \left[\frac{k}{(1 - k^2)}\right]^2 \tag{34}$$

where N is Avogadro's number, μ is the dipole induced by the crystal in the first layer of adsorbed vapor, R is the gas constant, k is a constant of zero dimensions, a is the polarizability of the adsorbed molecule, and a_1 is the spacing of the dipoles of the adsorbent. The exponent A in the K_3 term is the amount of adsorbed gas.

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The term K_3 is equal to K_2^b, where b is a constant. For use, Equation 32 is put into a log form and A is plotted against log log (p_o/p), and the result is a straight line.

Adsorption Kinetics

The adsorption of gases on solids may proceed at a fast rate which indicates surface adsorption on a crystal, or it may proceed at a much slower rate indicating penetration into the crystal. The adsorption of water vapor onto proteins is of the latter type, as has been shown for one protein, hemoglobin, by Eley and Leslie (1966). This study extended the observations to other systems with the same conclusions.

Studies of gas adsorption, particularly inorganic compounds, has shown that the process can not be described by simple mass action laws. The first investigators to study the problem quantitatively were Roginsky and Zeldovich (1934); they worked with the carbon monoxide - manganese dioxide system. They found that the kinetics could be represented by the equation

$$dq/dt = K \exp(-bq) \qquad (35)$$

where q is the amount of gas adsorbed, and K and b are constants. The equation states that the rate of adsorption possesses an activation energy which increases as the amount of the gas q adsorbed increases. This could be expressed as

$$dq/dt = K \exp(-\alpha q/RT) \qquad (36)$$

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where α / RT is the constant b in Equation 35. This equation is integrated to express q as a function of time, viz.,

$$q = (RT/\alpha) \left[\ln\{ t + (RT/\alpha k')\} - \ln (RT/\alpha k')\right] \quad (37)$$

where k' is a constant. This can also be written

$$q = (RT/\alpha) \ln (t + t_o) + \text{Constant} \quad (38)$$

Plotting q against $\ln (t + t_o)$ should give a straight line; t_o must be chosen empirically. If the value of t_o is too small, the line will be convex to the $\ln (t + t_o)$ axis, and if t_o is too large, the curve will be concave.

The number of adsorption sites alone is the limiting factor in the rate of gas adsorption; the amount of gas only determines the initial rate of the reaction. Taylor and Thom (1952) explain the kinetics by a mechanism of bimolecular site-site interactions. This kinetic law is given a different interpretation by Eley; his view was explained in the Introduction of this paper.

Electrolysis Rate

The evolution of hydrogen was calculated from Faraday's law of electrolysis, i.e., one Faraday of charge produces one equivalent of compound; diatomic hydrogen contains two equivalents so two Faradays are needed. From the fact that one ampere equals one coulomb/second, and that one Faraday is equal to 96,500 coulombs, one can calculate that 1 μamp. produces 5.18×10^{-12} (moles H_2/sec.).

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Using the equation $PV = nRT$, one can now calculate the pressure change since n , in (moles H_2/sec), is now known from Faraday's law. The system must be calibrated so that the volume V is known, of course.

The system used here had a volume of 422 $cm.^3$ so that the generation of hydrogen corresponded to 1.34×10^{-2} millitorr/μamp-min. The apparatus, when well out-gassed, had a sensitivity of about 10^{-9} moles of hydrogen.

III. EXPERIMENTAL METHODS

Samples

The materials used in this study were obtained commercially, with the exception of melanin, and they were not further purified. Each compound is described in more detail below.

(i) Hemoglobin was purchased from Gallard-Schlesinger and processed by Servac; the material was dialysed and twice recrystallized.

(ii) Cytochrome-c (horse heart) was obtained from the Sigma Chemical Company and was free of ammonium sulphate and sodium chloride. It was primarily in the oxidized form.

(iii) Collagen was obtained in the form of strips approximately 0.2 cm. wide and 5×10^{-3} cm. thick. The material was a special metal-free preparation from the Ethicon Corporation and was extracted from bovine Achilles tendon. Experimental cells of this material consisted of several strips side by side.

(iv) Melanin was prepared by an auto-oxidation polymerization of DOPA (dihydroxyphenylalanine). This material was extensively dialyzed, and

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it is the highest purity obtainable, and was prepared by Dr. Elliot Postow.

(v) Lecithin was purchased from Nutritional Biochemicals as synthetic B, γ-dipalmitoyl-DL-α-lecithin.

(vi) Deoxyribonucleic acid was purchased from the Sigma Chemical Company in the form of the sodium salt. It was extracted from salmon sperm.

Conductivity Experiments

Conductivity measurements were made on samples pressed into thin pellets. The die was pressed with the palm of the hand so that the pressure would not be great enough to denature the sample. These small pressures were found to be sufficient to provide samples with sufficient mechanical strength. The pellets were approximately one millimeter thick and were placed between two platinium foil electrodes. These were affixed to a Teflon block with screws. Before assembling the cell, the block was washed sequentially with ethanol, water, and again with ethanol; it was then only handled with forceps. The area of the electrodes was about 0.25 $cm.^2$. This sandwich cell was placed in a glass tube in the vacuum line. Electrical connections to the outside were made with tungsten feed-throughs sealed in quartz to minimize electrical leakage.

The potentials were varied from 1 volt applied (10 volts/cm.) to 300 volts applied (3,000 volts.cm.). All

measurements were d.c. In the case of some highly hydrated samples, polarization effects were large at the higher voltages, and it was necessary to follow the electrometer readings with a strip chart recorder to determine the "equilibrium current" - this often required 3 hours.

Adsorption Isotherms

Powder samples were placed in the Cahn Vacuum Microbalance (Model RG) which was counterweighted so that the sensitivity was increased. The hang-down tube of the balance was thermostated by circulating water in an outer jacket. The temperature was kept at $26.0^{\circ} \pm 0.1\ ^{\circ}C$.

All samples were heated prior to the start of the experiments with either a heating tape or a heat lamp to determine the dry weight. The temperature was about $75^{\circ}C$. At this temperature, the sample could be brought to constant weight (less than a change of 0.02%) within two hours. On some samples, pyrolysis would occur if heated to higher temperatures - this was very true of collagen.

While the samples were being heated, the system was evacuated to a pressure of approximately 5 millitorr as measured on an ionization gauge. While the system was being pumped on, the traps were always cooled with dry ice to prevent back streaming of oil which could coat the samples and affect the adsorption.

Water vapor was introduced from side tubes containing vacuum degassed water. The vapor pressure was determined by means of a Dubrovin ("cartesan diver") gauge which had

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a least count of 0.1 torr and a range of from 0.0 to 10 torr. Above this limit, the temperature of the water in the side tube was varied to control the pressure. The water temperature could be measured to ± 0.1 C.° and the vapor pressure could be found to three significant figures from the Handbook of Chemistry and Physics. This proceedure was found to be superior to the measurement of pressure directly with the mercury manometer as this device could only be read to ± 1.0 torr.

The Cahn RG was connected to a strip-chart recorder to determine more easily the adsorption kinetics and the equilibrium weight. The balance pans were counter-weighted so that the full-scale measurement was 10.0 mg. The sensitivity of the balance at this setting was such that a weight change of five micrograms could be detected; this was 0.01% of the sample weight.

Bouyancy effects could be calculated from the equation

$$\beta = P M W / \rho R T \qquad (39)$$

where P is the gas pressure (atm), M is the molecular weight of the gas, W is the weight of the sample, ρ is the density of the sample, and R and T are the gas constant and the absolute temperature, respectively. It was found, however, that even at saturation vapor pressure, the bouyancy correction for water was only one microgram. Therefore,in this work, no bouyancy corrections were used.

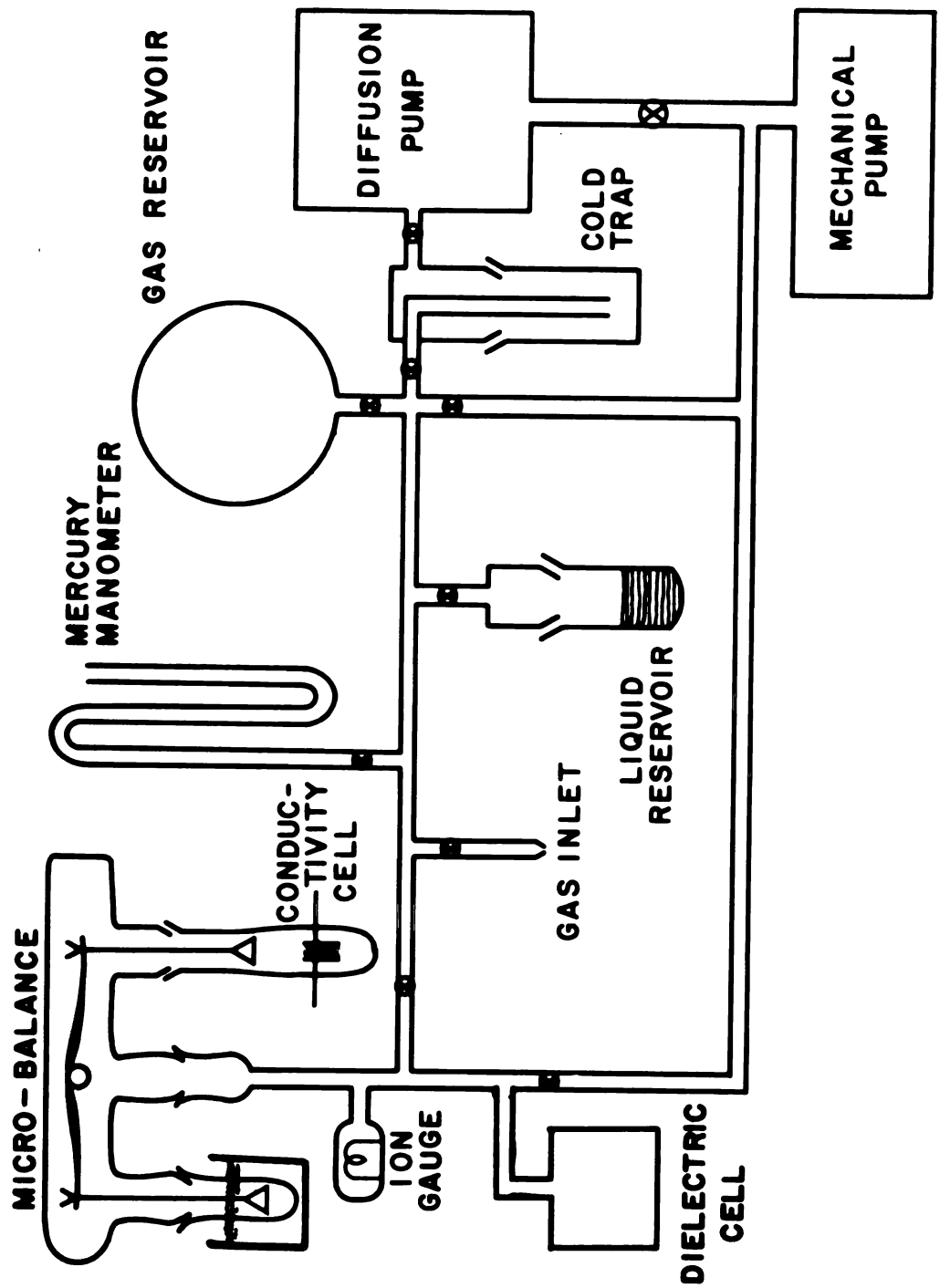

Figure 2. Schematic of vacuum-microbalance apparatus.

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Equilibrium was generally reached in less than six hours for a p/p_o of from 0.0 to 0.4; equilibrium was defined as no detectable weight change after three hours (note that weight changes of 0.01% were detectable). The higher values of p/p_o always required longer periods to reach equilibrium, and the measurements were made after a period of 24 hours. The time to reach equilibrium was less in the instances where powder samples were used as compared to pressed pellets. This is, no doubt, the result of easier diffusion of the water vapor. In that equilibrium weights were not affected by the sample form, powder samples were used to reduce the time needed for each weight determination.

Adsorption Kinetics

Using the above mentioned Cahn RG microbalance and a recorder, the adsorption kinetics could be studied. In these instances, it was necessary to bring the sample to its dry weight before commencing the adsorption. Sample weights and times were read directly from the strip chart.

Dielectric Measurements

The measurements were made on pressed pellets 1 cm. in diameter and about 1 mm. thick. The samples were slowly pressed to avoid denaturation to a pressure of 10^3 Kg./cm.^{2}. They were placed in a Teflon insulated stainless steel dielectric cell (Balsbaugh Laboratories - Model LD-3).

The dielectric cell was placed in a large, brass vacuum

tight chamber (grounded) and attached to the vacuum line. Water vapor pressure could be determined in the same way as described under "Adsorption Isotherms".

In order to insure that equilibrium had been reached, twenty-four hours intervened between the introduction of water vapor and the dielectric measurements. This was found to be sufficient as equilibrium times for these experiments was similar to water adsorption equilibrium times.

Measurements were made in the frequency range 100 Hz. to 10^5 Hz. with a General Radio 1610-B Capacitance Measuring Assembly which consisted of:

(i) A Schering Bridge (716) which reads from 30 Hz. to 10^5 Hz.

(ii) A Guard Circuit (716-P4)

(iii) An Oscillator (1302-A) with a range of 10 Hz. to 10^5 Hz.

(iv) An Amplifier and Null Detector (1231-B)

Denaturation

Hemoglobin was the only compound which was tested for denaturation, however, the proceedure could only discover the grossest types. Samples of hemoglobin were weighed, dissolved in distilled water, and filtered through Millipore filters. The filter was dried in an oven and weighed to determine the water-insoluable protein.

The average of the two samples tested was 5.8% denaturation.

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Electrolysis Experiments

The samples for electrolysis were prepared and mounted in a manner similar to that described under "Conductivity Measurements". The samples were suspended in glass sample chambers, care being taken to avoid contact with the walls. This was easily accomplished when the chambers were attached to the vacuum line, Figure 3, by simply tilting them. The platinium electrodes were blocking for protons so all protonic carriers were released as hydrogen gas.

The glass sample chambers were made without glass joints and were closed with a torch when the samples were added. Thus no hydrogen was able to leak into or out of the system through the vacuum grease.

The system was pumped with a fore pump and a glass three-stage oil diffusion pump, and the system pressure (exclusive of the mercury vapor from the McLeod gauge) was less than 5×10^{-6} torr. All samples were pumped continuously from the time they were sealed to the line except for the time of electrolysis and measurement. In no case were they again exposed to atmospheric pressure.

Many designs were tried before the present one was arrived at, and it represented the best compromise of sensitivity, precision , and accuracy. One other design is described more fully in a later section.

The volume of the electrolysis manifold was measured using a mercury manometer, a calibrated volume, and Boyle's law. In this way, the volume was determined to 1%.

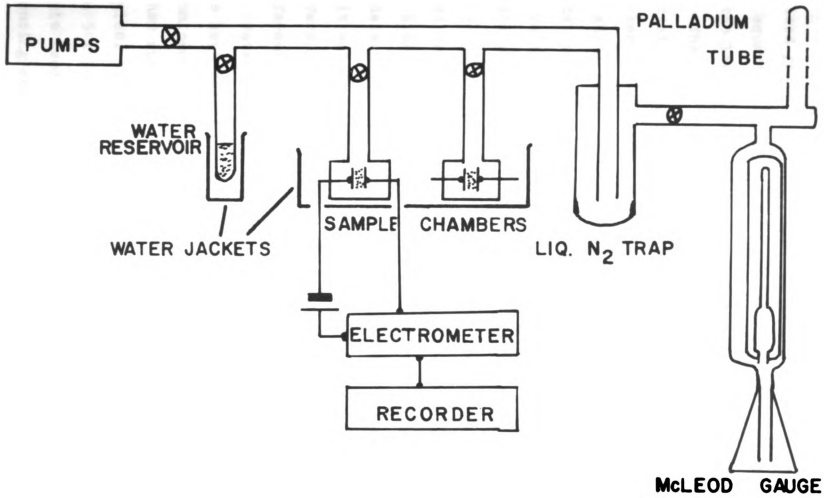


Figure 3. Diagram of the electrolysis apparatus.

The number of moles of hydrogen generated could be determined from a measurement of the pressure with the McLeod gauge and a knowledge of the manifold volume and the equation $PV = nRT$; the gas laws were assumed to hold at the low pressures involved, viz., 0.1 to 50 millitorr.

The sample chambers were immersed in a water bath held at the same temperature at which the adsorption isotherms were made (26.0° C.). The samples were hydrated for about three hours (sufficient for electrical measurements) by opening the stopcock to the water reservoir. The vapor pressure was changed by adjusting the temperature of the water in the reservoir; this was done with a separate circulating water bath. The hydration state was then determined from the adsorption isotherm. The samples used in the electrolysis were from the same bottle as those used in determining the adsorption isotherm; this was necessary as it was found that there were small, but significant, differences between adsorption isotherms of samples from different lots.

Potential differences for the electrolysis experiments were kept between 50 and 200 volts, as it was found that erronious results could arise from the application of higher applied potentials, especially with the drier samples. The currents passed with these potentials varied from 5×10^{-8} amperes to 5×10^{-4} amperes depending on the sample used and its hydration state. Hydration states producing currents of less than 10^{-8} amperes (at 200 v.)

could not be studied in this apparatus as they produced to little hydrogen to be detected against the "background" pressure. This "background"was the result of out-gassing of the walls of the sample chamber, the sample itself, and the tubing of the McLeod gauge, none of which could be heated; the "background" pressure was on the order of 0.1 millitorr.

The number of coulombs passed was determined graphically with an electrometer and a strip-chart recorder. In those cases where polarization effects produced a rapid change of the current with time, a mechanical integrator attached to the recorder was used. The number of coulombs passed could be determined to about 2%.

With a knowledge of the number of coulombs passed, the theoretical yield of hydrogen could be determined, assuming 100% ionic conduction, from the laws of electrolysis. Thus, two equivalents of charge are needed to produce one mole of diatomic hydrogen.

The amount of hydrogen evolved during electrolysis was measured by first measuring the pressure of hydrogen plus residual gas (nitrogen and oxygen), and then heating the palladium tube to allow the hydrogen to leak out. Palladium is specifically permeable to hydrogen. The hydrogen removal generally took about 20 to 30 minutes. The second pressure measurement then determined the remaining residual gas. The hydrogen pressure was the difference between the first and second measurement. Because of the

specific permeability of palladium, no chemical analysis for hydrogen was needed.

It was necessary to use a cold trap during the measurements to remove the water vapor. If this was not done, it would condense in the McLeod gauge upon compression of the gas with the mercury. Using liquid nitrogen helped to reduce the residual gas pressure somewhat.

Unlike ionization gauges, a McLeod gauge does not need a specific calibration for hydrogen thus all measurements were "absolute". To insure that there was no loss of hydrogen from the system, preliminary tests were made using oxalic acid dihydrate. This compound was pressed into pellets and mounted in a similar manner to the biological samples and hydrated. It is strongly suspected that these crystals are protonic conductors from the work of Pollack and Ubbelohde (1956) on the activation energy and conductivity. Two measurements on hydrated samples ($p/p_o = 0.82$) gave values of 98% and 112%. The average was 105%, and the difference between the two values, 14%, served as an indication of the reproducibility in the other measurements.

A sheet of Teflon was also placed into the sandwich cell, and the chamber was fully hydrated ($p/p_o = 1.0$). The leakage current was 10^{-10} amperes at 500 volts; this was 10^{-6} of the current which would pass through a protein sample at this same hydration and potential.

Graphite, in the form of Aqua Dag was placed in the sample holder, hydrated, and electrolysed. A current of

300 milliamperes was passed for 20 minutes with no detectable release of hydrogen. This was to be expected in that graphite is an electronic conductor.

Tracer Experiments

The amount of protonic conduction in hydrated compounds was examined by a tracer technique using tritiated water. The apparatus is shown in Figure 4. It was used both for the calibration of the water activity and the electrolysis of the biomacromolecules.

The device consisted of a sample holder and chamber similar to that described earlier, and an evacuated manifold. For the calibration of the activity of the water, a small Teflon spacer was placed in the sandwich cell to prevent the electrodes from short circuiting and to hold the water sample.

The tritiated water was calibrated by determining the counts per minute produced per microampere-minute (cpm/μamp-min.) for a series of dilutions. The change in activity was found to be linear with concentration. By extrapolating back to zero dilution, it was possible to determine the activity of the original tritiated water sample. Undiluted water was too radioactive to determine directly.

The activity of the water was found to be independent of the pH of the water used for dilution.

The activity measurements, and electrolysis measurements were made by evacuating the entire manifold, and then

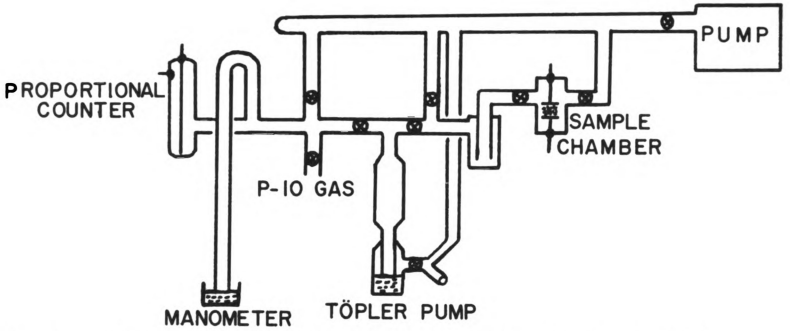


Figure 4. Diagram of the apparatus used in the tritium tracer experiment.

closing off the sample chamber while hydration and electrolysis were occuring. At the conclusison of the electrolysis, the tritium evolved was separated from the tritiated water vapor by pumping the gas and vapor through a liquid nitrogen cooled trap with a Toepler pump. The tritium was pumped into the counter tube, and after ten cycles of the pump, more than 99% of the tritium was in the proportional counter tube.

With the tritium in the counter tube, one atmosphere of P-10 gas (argon, 10% methane) was admitted rapidly to prevent the back diffusion of tritium. The activity of the sample (in counts per minute) was then measured with the scaler.

The counter tube was of an all glass design for the outer tube. The inner surface was grounded while the inner tungsten wire (2 mil) was at a potential of +2,100 volts. The plateau region of the counter was from 2,000 volts to 2,300 volts.

At the end of the counting period, the experiment was terminated by pumping out all of the gas. All counting experiments were bracketed by measurements of the background. This was typically of from 100 cpm. to 300 cpm. If there was no leakage of the tritiated water vapor past the cold trap, the background after the experiment was the same as before the electrolysis began. However leakage, when it occurred, could always be easily seen in that the background after electrolysis would be several thousand cpm.

In addition, it would not fall unless there was protracted pumping for two days with heating. In measurements where no leakage occurred, the background was the same as initially found with only short pumping times - on the order of one to two minutes.

The same proceedure was used for the electrolysis of the sample as was followed in the calibration of the water activity. Thus if any loss occurred through leakage or adsorption, this would be true for the calibration and would simply result in a lower calibrated activity.

The loss in activity of the radioactive water from the exchange of tritium with the labile protons of the protein was measured by two methods. In the first, the sample (hemoglobin) was hydrated in a U-tube (water on one side and protein on the other) for five days. At the end of this period, the water was desorbed from the protein by placing one side of the tube in a dry ice bath; this water was then diluted and analysed for its activity. The loss in activity represented the amount of radioactivity taken up by the protein by exchange of protium for tritium.

In the second method, the sample (collagen) was hydrated and then dehydrated after a period of about six hours, the time needed for electrolysis. The sample was then placed in a large volume of water so that the tritium on the collagen would exchange for protium of the water. The water was then analysed for activity.

These effects are mentioned in that the adsorption and exchange are the most bothersome parts of this method.

IV. EXPERIMENTAL RESULTS

Adsorption Studies

It can be seen from the adsorption isotherms in Figures 5 and 6 that all of the compounds form the BET (Type II) isotherm. This is common for compounds displaying physical adsorption as contrasted with chemisorption.

With the exception of melanin, the compounds did not exhibit a hysteresis when dried and rehydrated. It was found that two states of melanin appear to exist, viz., a "low adsorption" and a "high adsorption" state. If the melanin was dried at 75° C. and hydrated at 26°C., a given hydration would be found. Then if the samples were pumped "dry", but not heated, it was found that upon hydration at 26° C. to the same value of p/p_o, a larger amount of water was adsorbed. This effect was experimentally disconcerting at first, and the reason for the "activation" is not known.

Excessive heating of the sample will alter the adsorption isotherm to a large degree. This was found to be true for collagen; the amount of water adsorbed was considerably much less when the sample had been heated so that slight pyrolysis had occurred as evidenced by a slight darkening of the strips.

Figure 5. Adsorption isotherm of water on melanin, collagen, and salmon sperm DNA at 26.0° C. The solid circles are melanin, the open circles are DNA, and the squares are collagen.

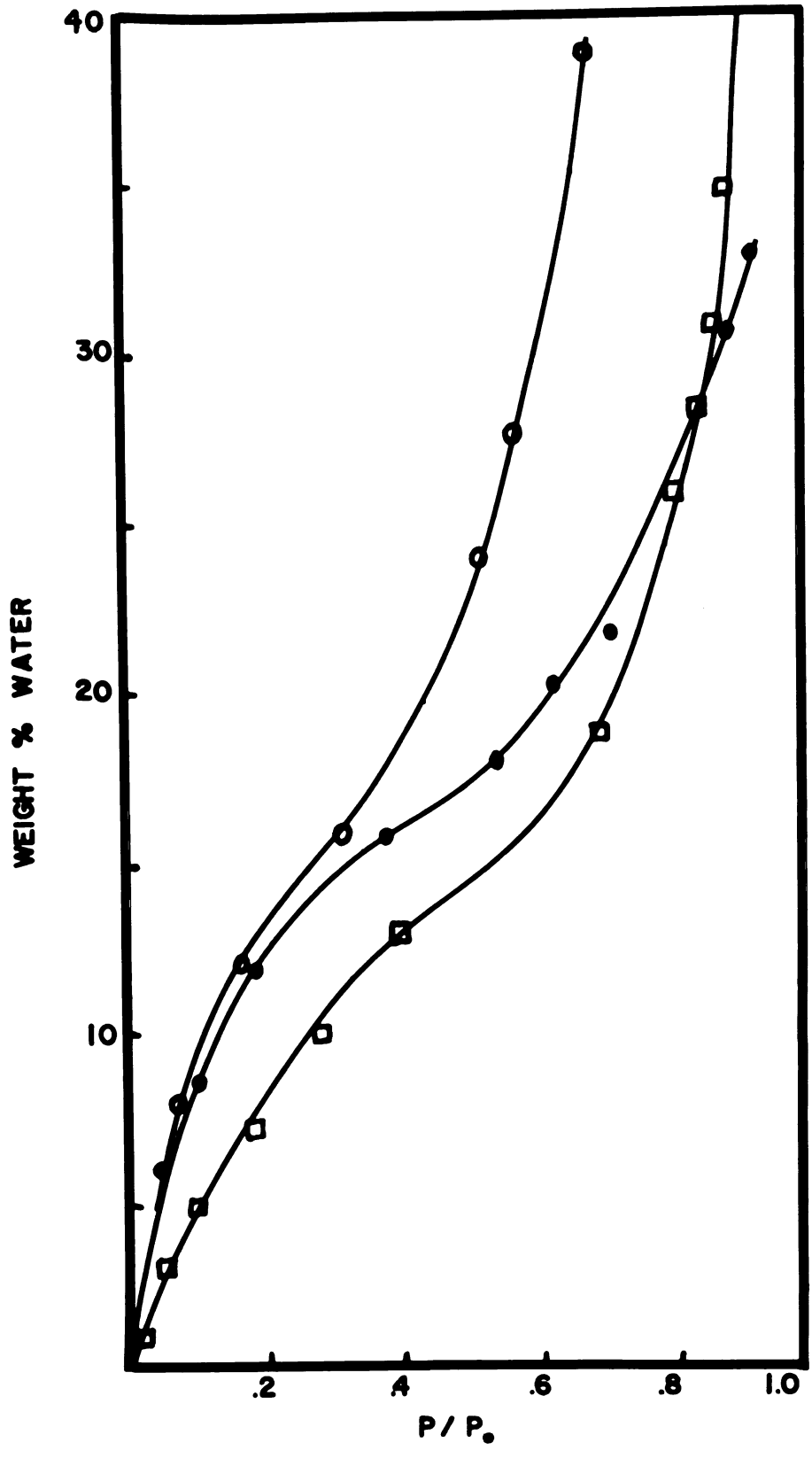
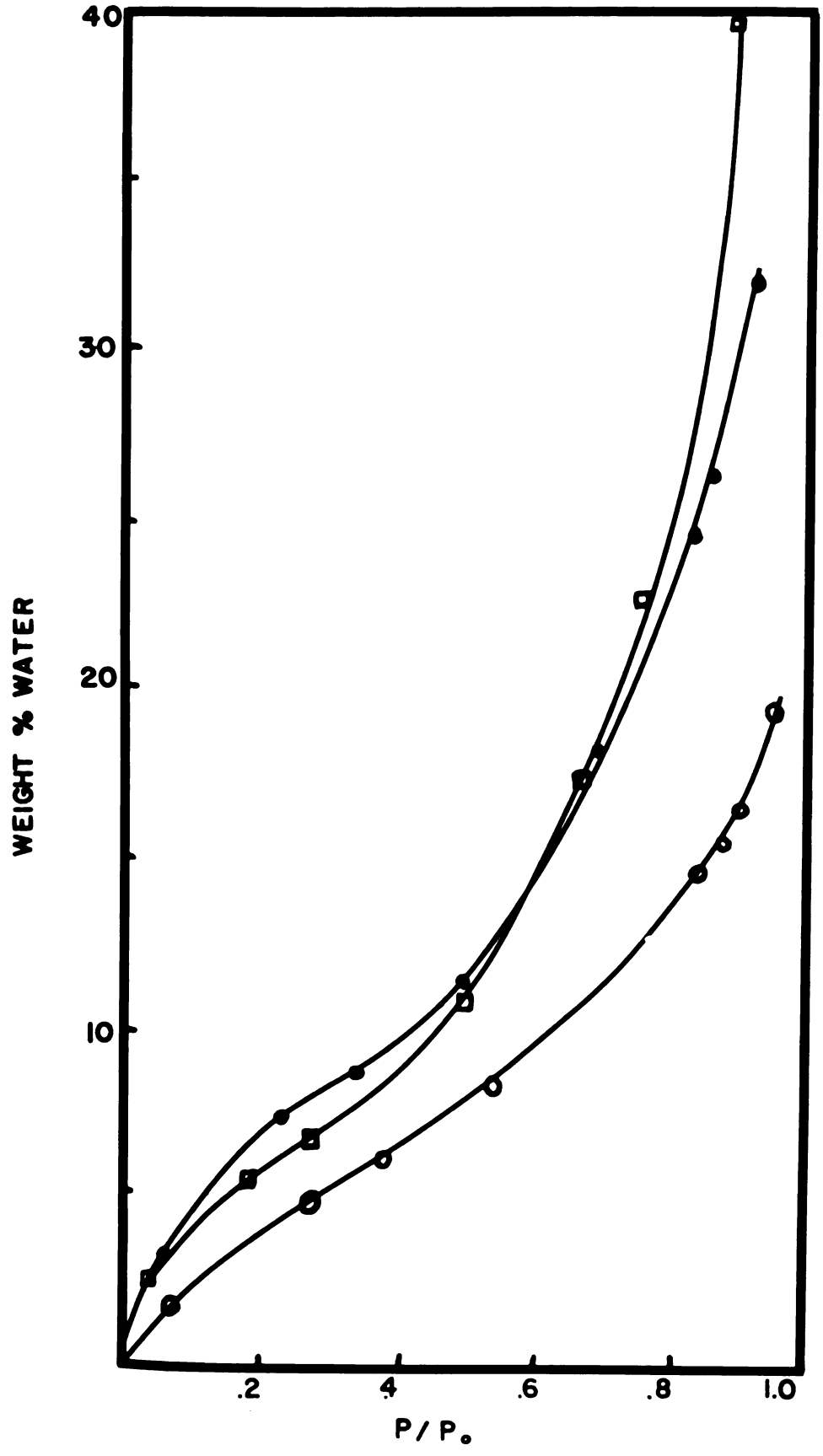

40
30
20
10
WEIGHT % WATER
.2
.4
.6
.8
1.0
P / P₀

Figure 6. Adsorption isotherm of water on lecithin, cytochrome-c, and hemoglobin at 26.0° C. The solid circles are the hemoglobin, the open circles are the lecithin, and the squares are the cytochrome-c.


40
30
20
10
WEIGHT % WATER
.2
.4
.6
.8
1.0
P/P₀

Sample characteristics are also changed if the samples are hydrated to such an extent that they dissolve in their water of hydration. Thus a sample of cytochrome-c was found to display entirely different adsorption characteristics after excessive wetting and redrying in the balance.

It is necessary to follow the same precautions in the electrolysis cells as followed in the adsorption isotherm cells as the latter must serve as a calibration for the former. This is particularly true of heating effects.

BET plots were made of the adsorption data, and these are shown in Figures 7 and 8. They are linear to a value of p/p_o of about 0.6. From these plots, a value may be found for v_m, the monolayer coverage. These values of the monolayer coverage are shown in Table 1. In addition, the values found by Postow (1968) and Cardew and Eley (1958) for hemoglobin are given here for comparison.

Bradley adsorption isotherms were plotted in the hope that they would provide a method of determining the amount of adsorption with a minimum number of measurements. These are shown in Figures 9 to 12. Unfortunately the isotherms are not linear over the entire range of p/p_o for half of the materials tested, and breakpoints were noted. Its range of linearity, however, is considerably wider than the BET plots.

Kinetic Studies

The adsorption kinetics of four of the compounds were studied, with the results that all could be fitted to the

Figure 7. BET plot of water adsorption on lecithin, cytochrome-c, and hemoglobin at 26.0° C. The solid circles are hemoglobin, the open circles are lecithin, and the triangles are cytochrome-c.

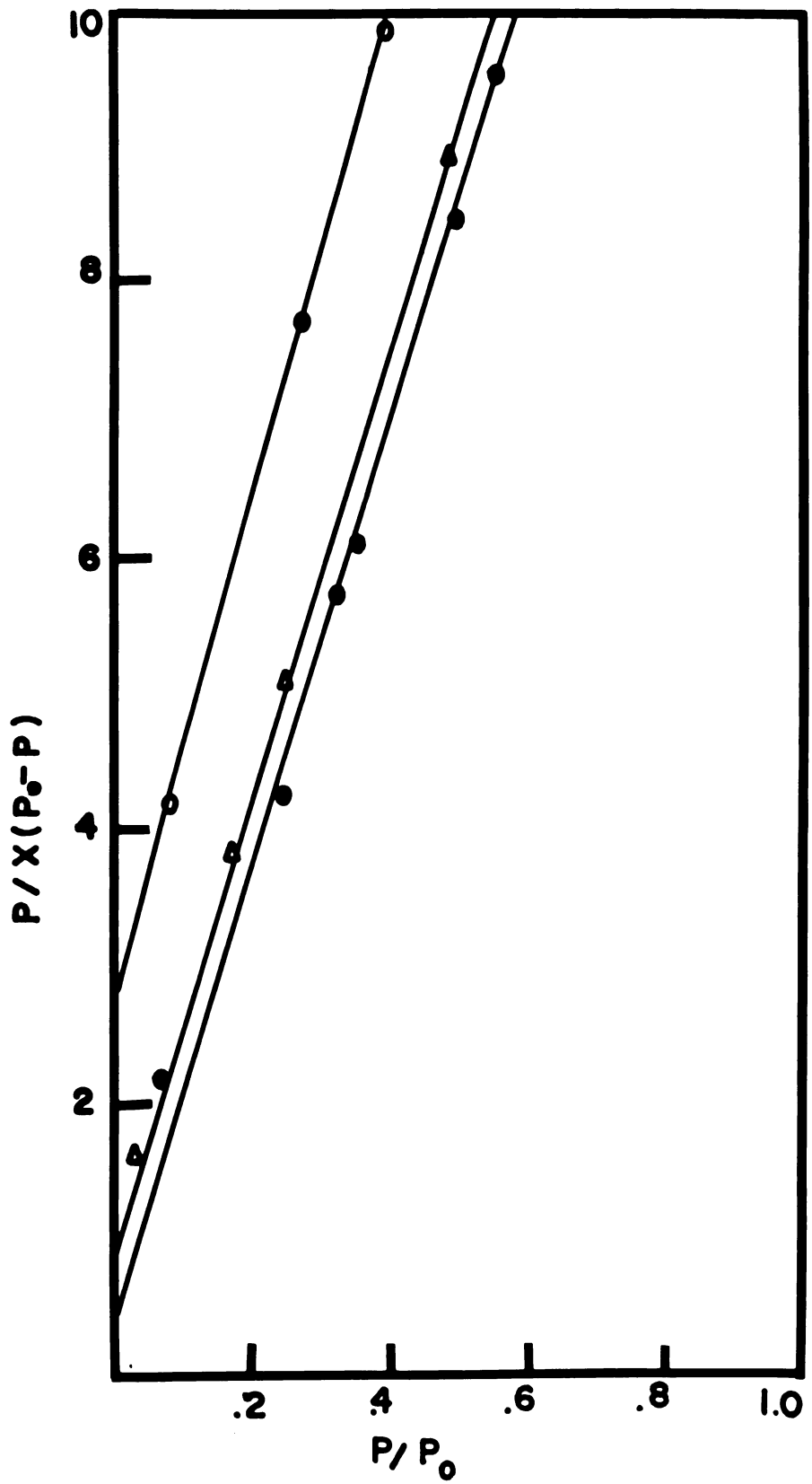

10
8
6
4
2
P/X(P₀-P)
.2
.4
.6
.8
1.0
P/P₀

Figure 8. BET plot of water adsorption on collagen, melanin, and salmon sperm DNA at 26.0° C. The solid circles are collagen, the open circles are DNA, and the triangles are melanin.

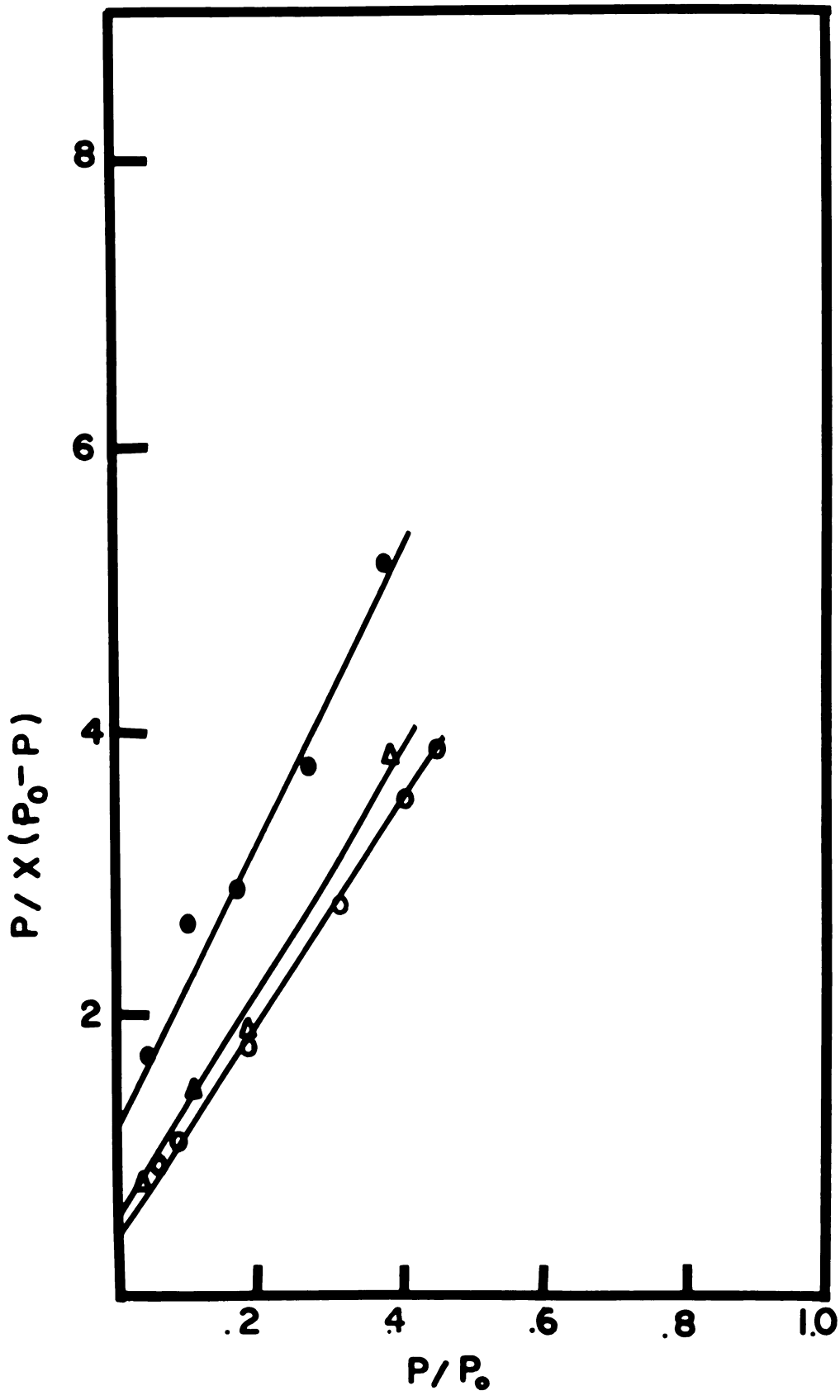

8
6
4
2
P/X(P₀-P)
.2
.4
.6
.8
1.0
P/P₀

Table 1. The BET monolayer coverage for the materials tested in this work.

Material	BET Monolayer
I. By Powell	
Collagen (@26° C.) + water	7.9 %
Salmon Sperm DNA "	12.4 %
Hemoglobin "	6.1 %
Melanin "	12.4 %
Lecithin "	4.6 %
Cytochrome-c "	6.3 %
II. By Postow (1968)	
Hemoglobin (@ 23° C.) + water	8.5 %
Hemoglobin + methanol	13.5 %
Hemoglobin + ethanol	7.5 %
III. By Cardew and Eley (1958)	
Hemoglobin (@ 30° C.) + water	5.76%
Hemoglobin (@ 40° C.) + water	5.72%

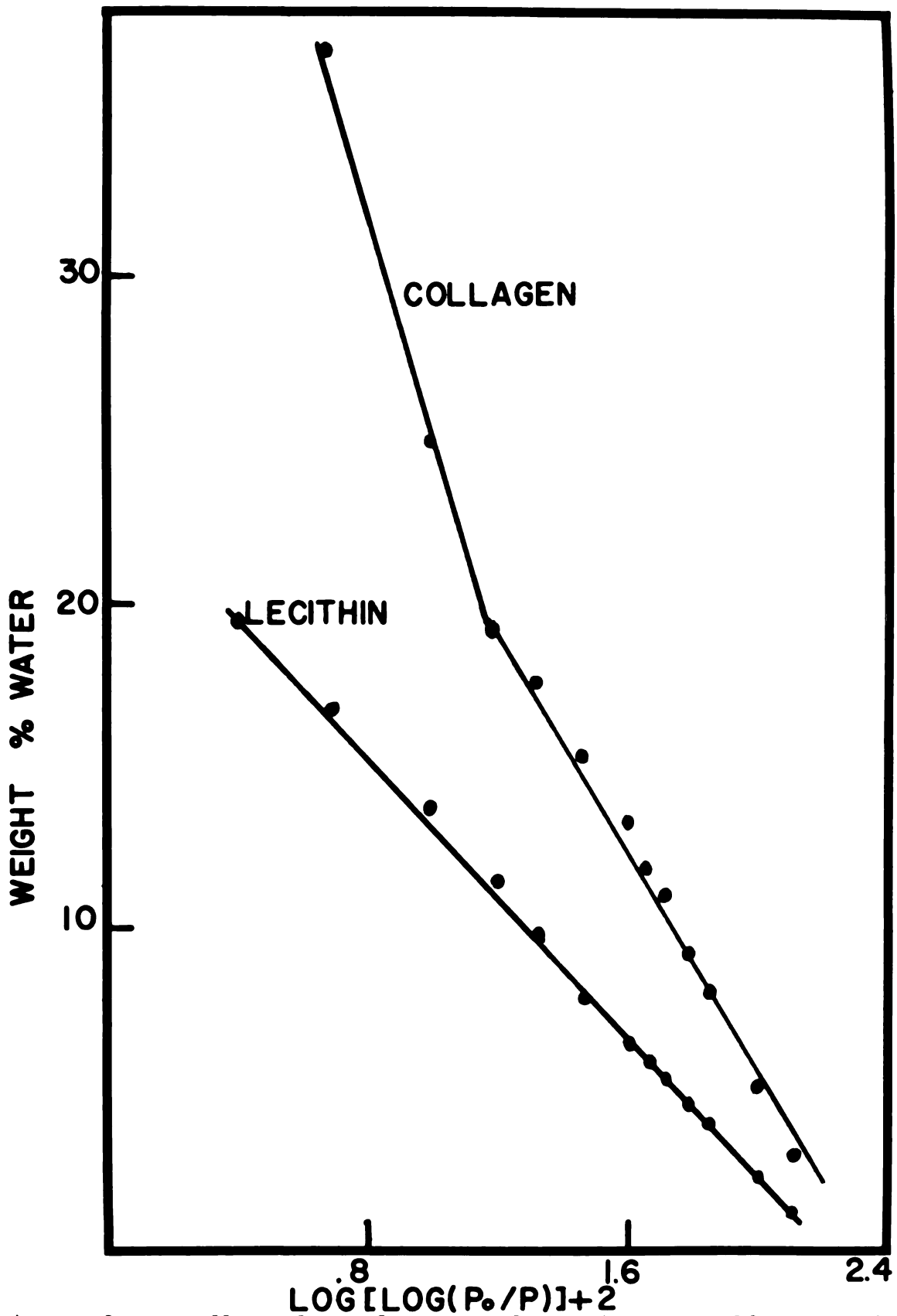


Figure 9. Bradley plot of water adsorption on collagen and lecithin

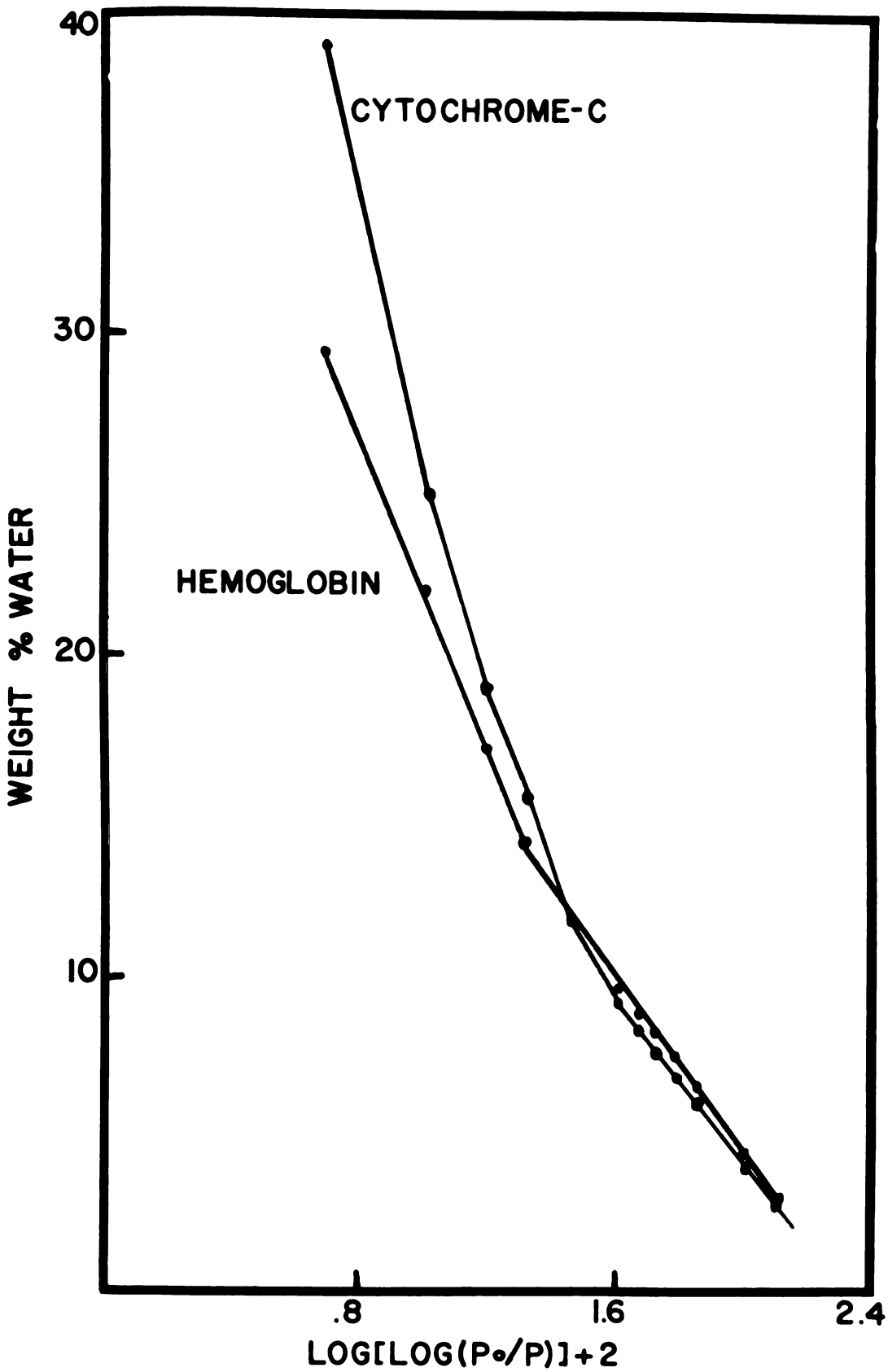


Figure 10. Bradley plot of water adsorption on hemoglobin and cytochrome-c.

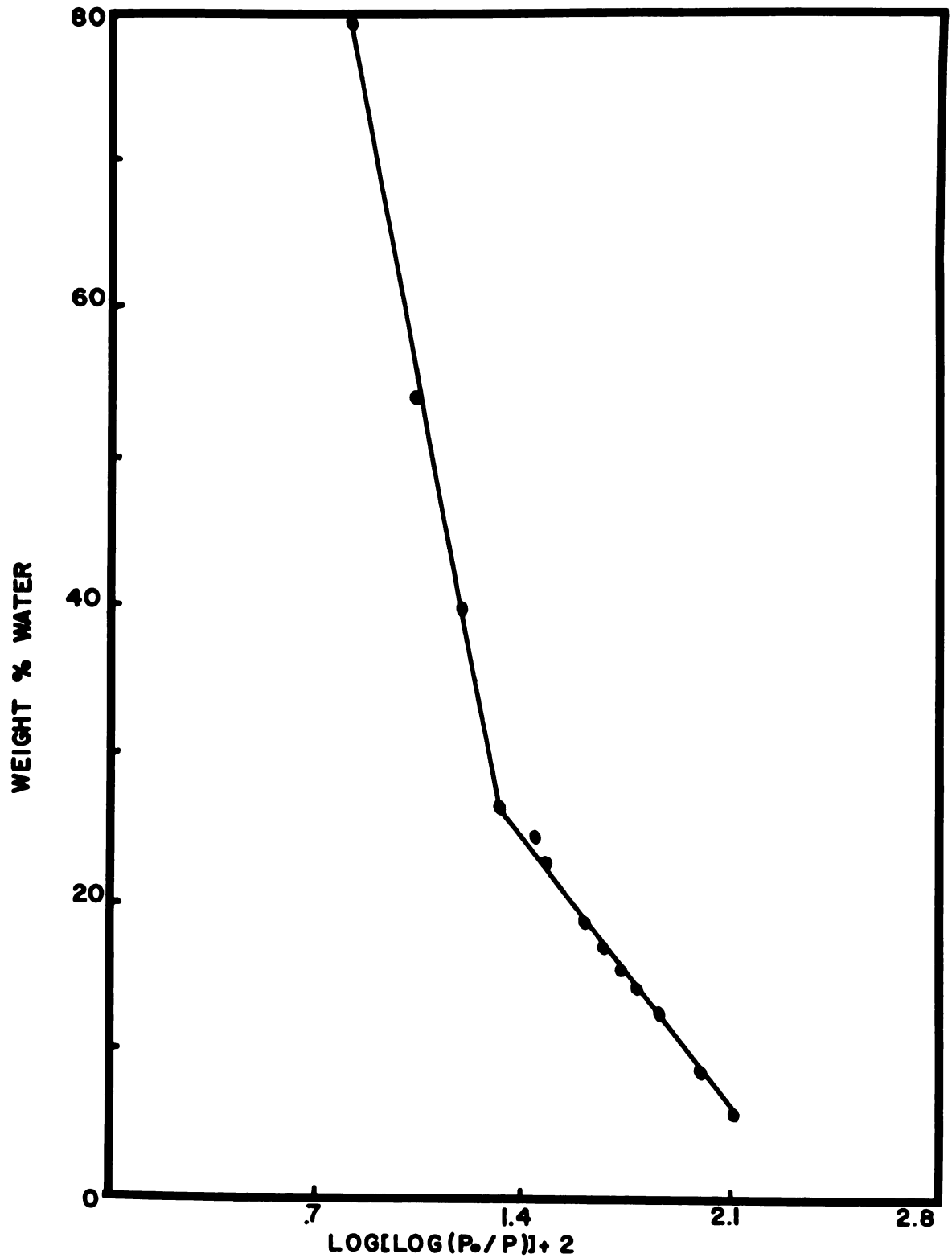


Figure 11. Bradley plot of water adsorption on DNA.

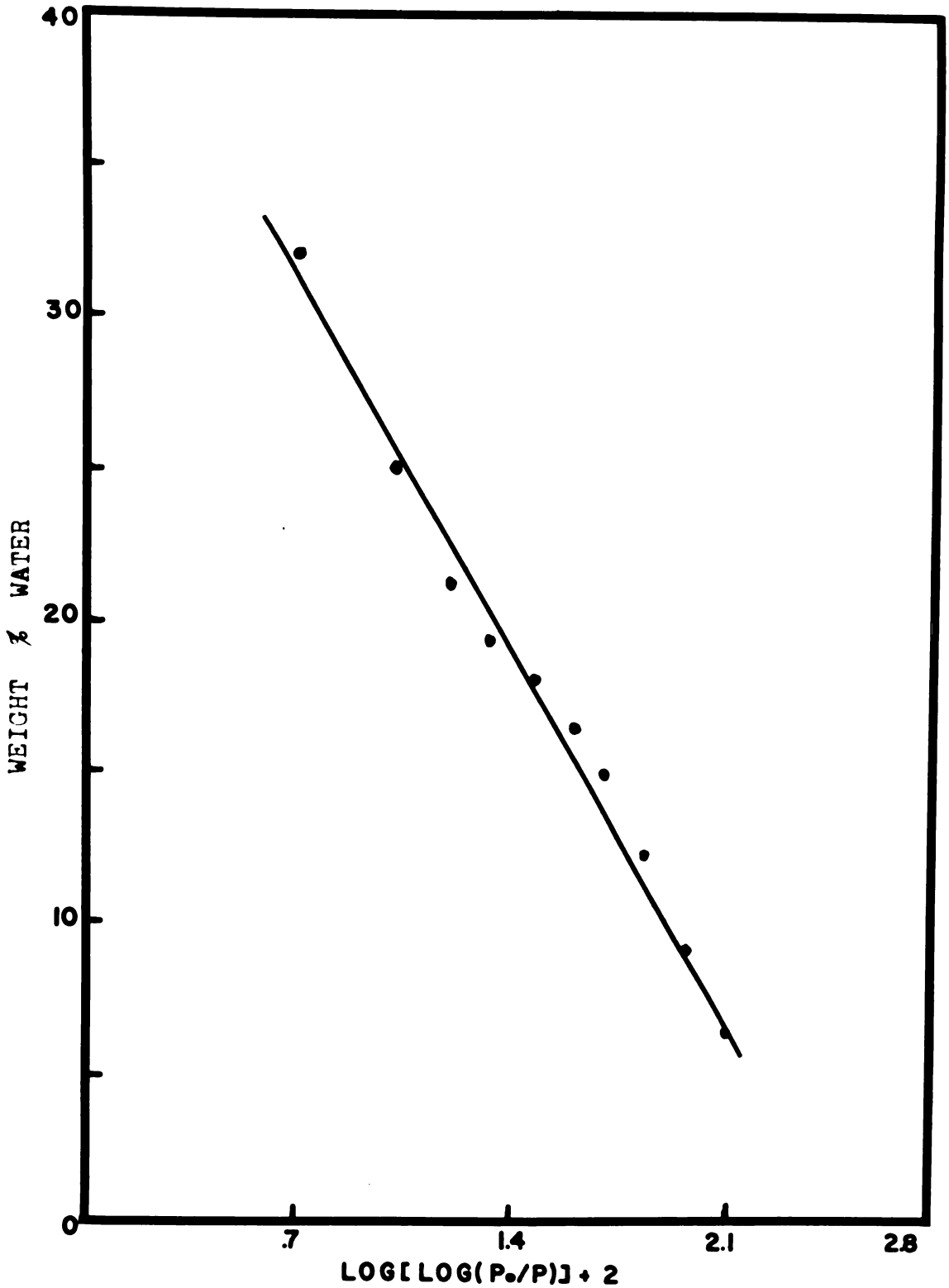


Figure 12. Bradley plot of water adsorption on melanin.

Roginsky-Zeldovich, or Elovich, equation. This data is shown in the Figures 13 to 16. The free parameter, t_o, is indicated at the side of each line. It will be seen that all required a small value of t_o. This value must always be positive.

It was observed that the conductivity increase does not follow Roginsky-Zeldovich kinetics. If one compares the conductivity at any one given point in time during the adsorption process, the "equilibrium weight" can be found from a graph of conductivity vs. equilibrium weight. The values from the graph correspond to conductivities and weights at equilibrium, i.e., after 24 hours.

It can be seen from Figure 14 that the"current weight" does not follow the Elovich equation. A curve which is concave to the right can not be made linear as only positive values of t_o are allowed.

Conductivity Measurements

All compounds tested were found to give conductivity increases with hydration. This effect is shown in Figures 17 to 23. The conductivity increases could be fitted to the equation

$$\ln \sigma = \alpha m + \ln \sigma_{dry} \qquad (40)$$

Additionally, all samples showed a saturation of current with increasing hydration with the exception of hemoglobin+ water and hemoglobin+methanol. Saturation occurred after the adsorption of about three BET monolayers. This was as

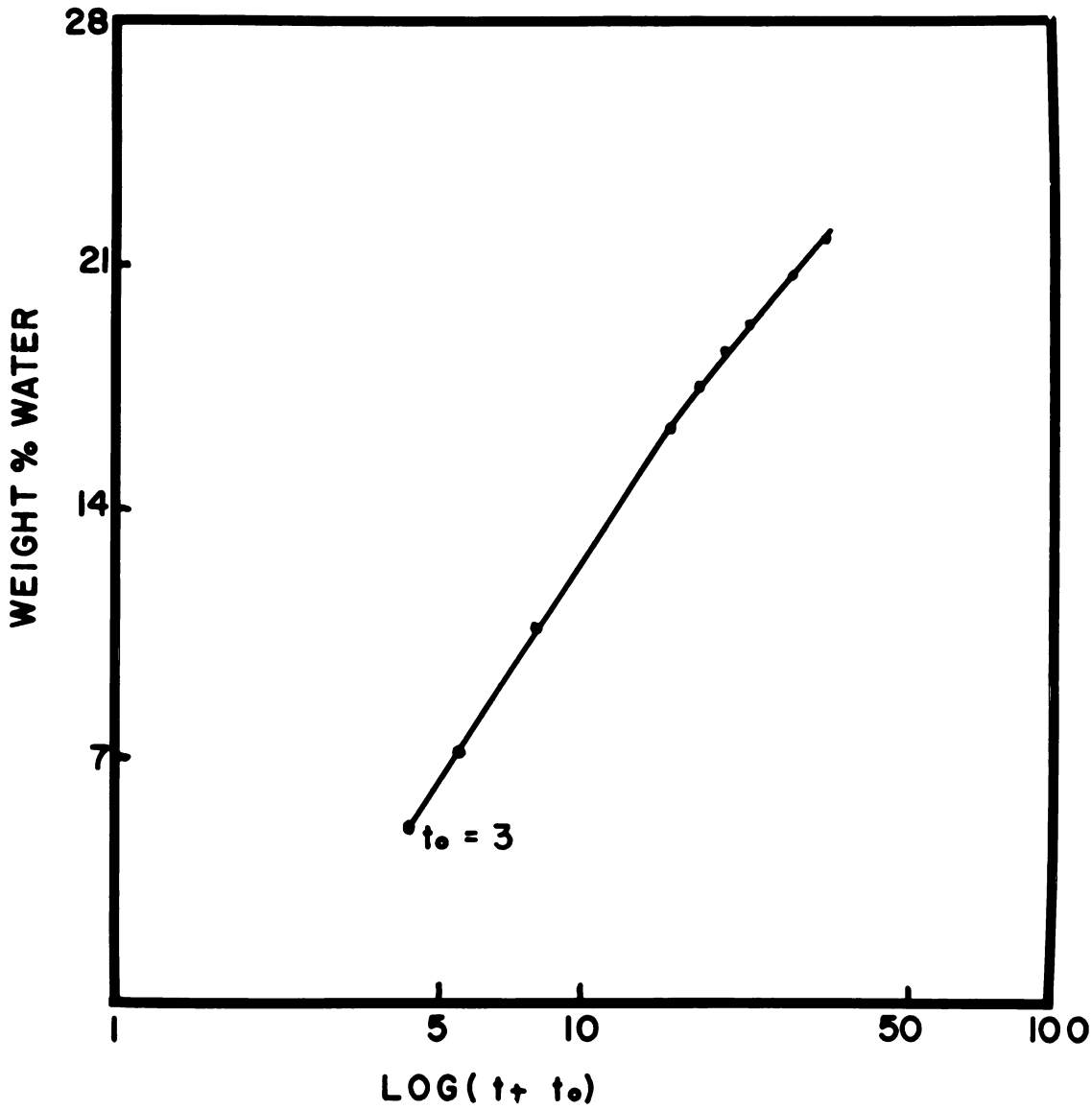


Figure 13. Adsorption kinetics of water on hemoglobin.

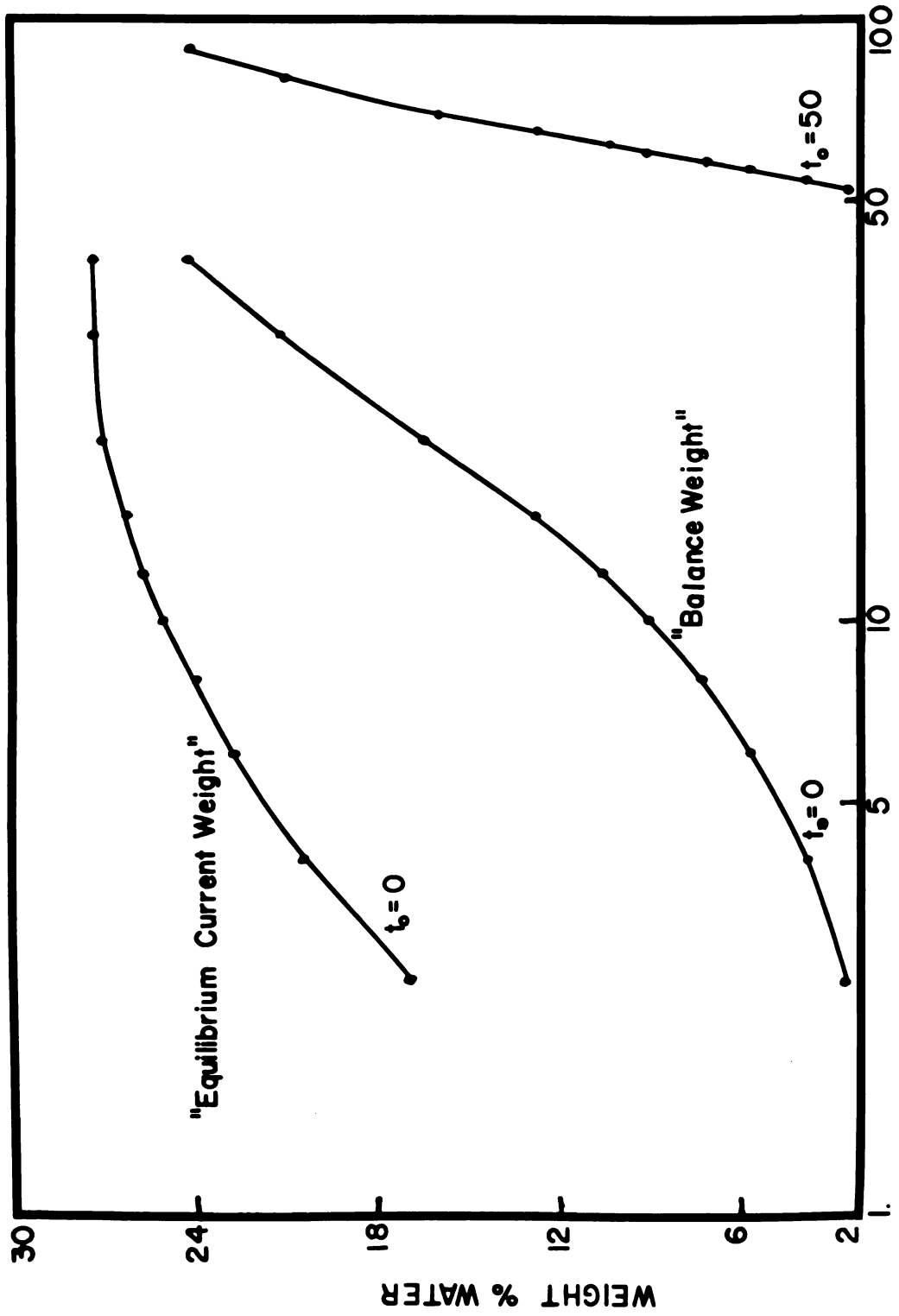


Figure 14. Adsorption kinetics of water on collagen.

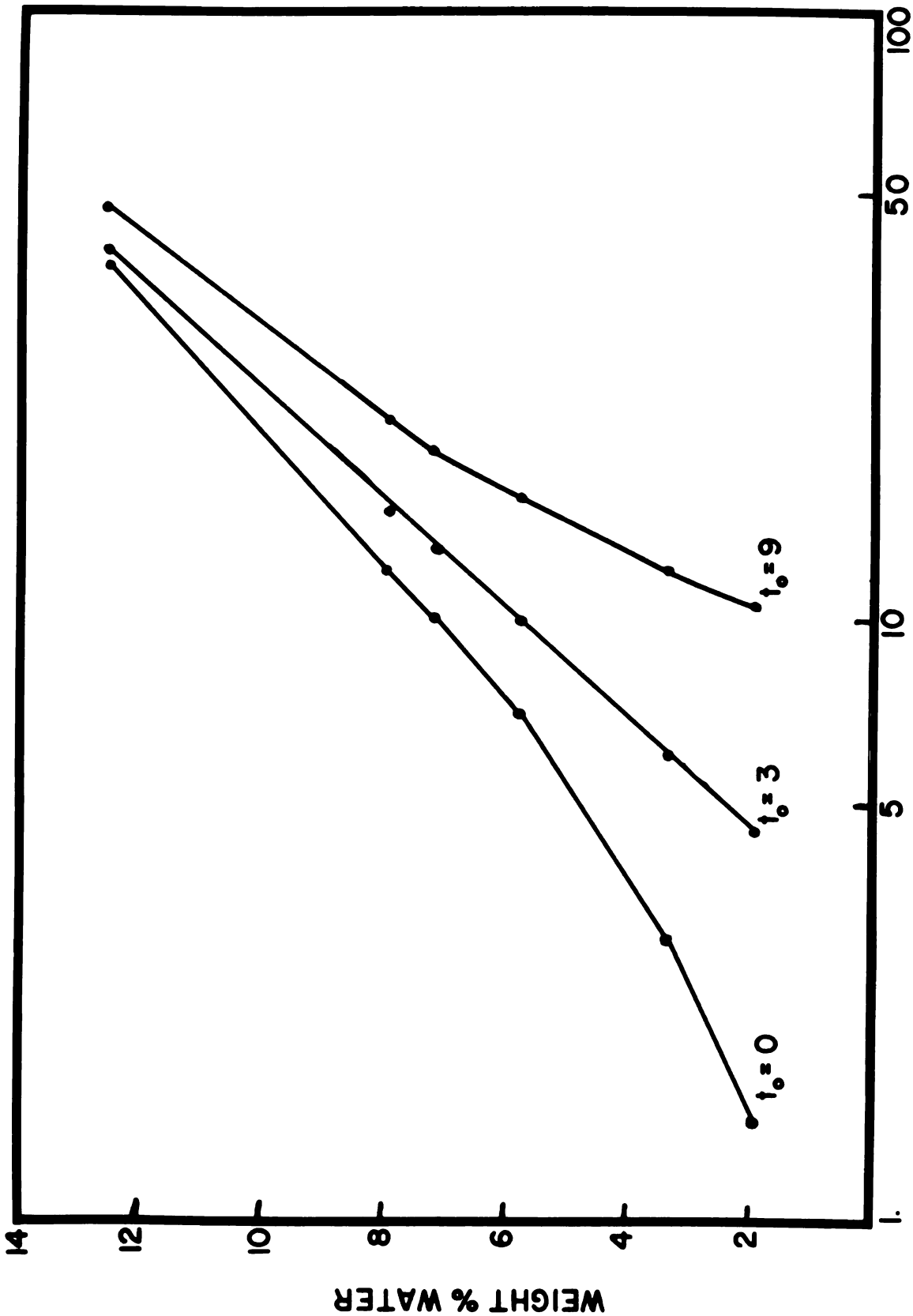


Figure 15. Adsorption kinetics of water on lecithin.

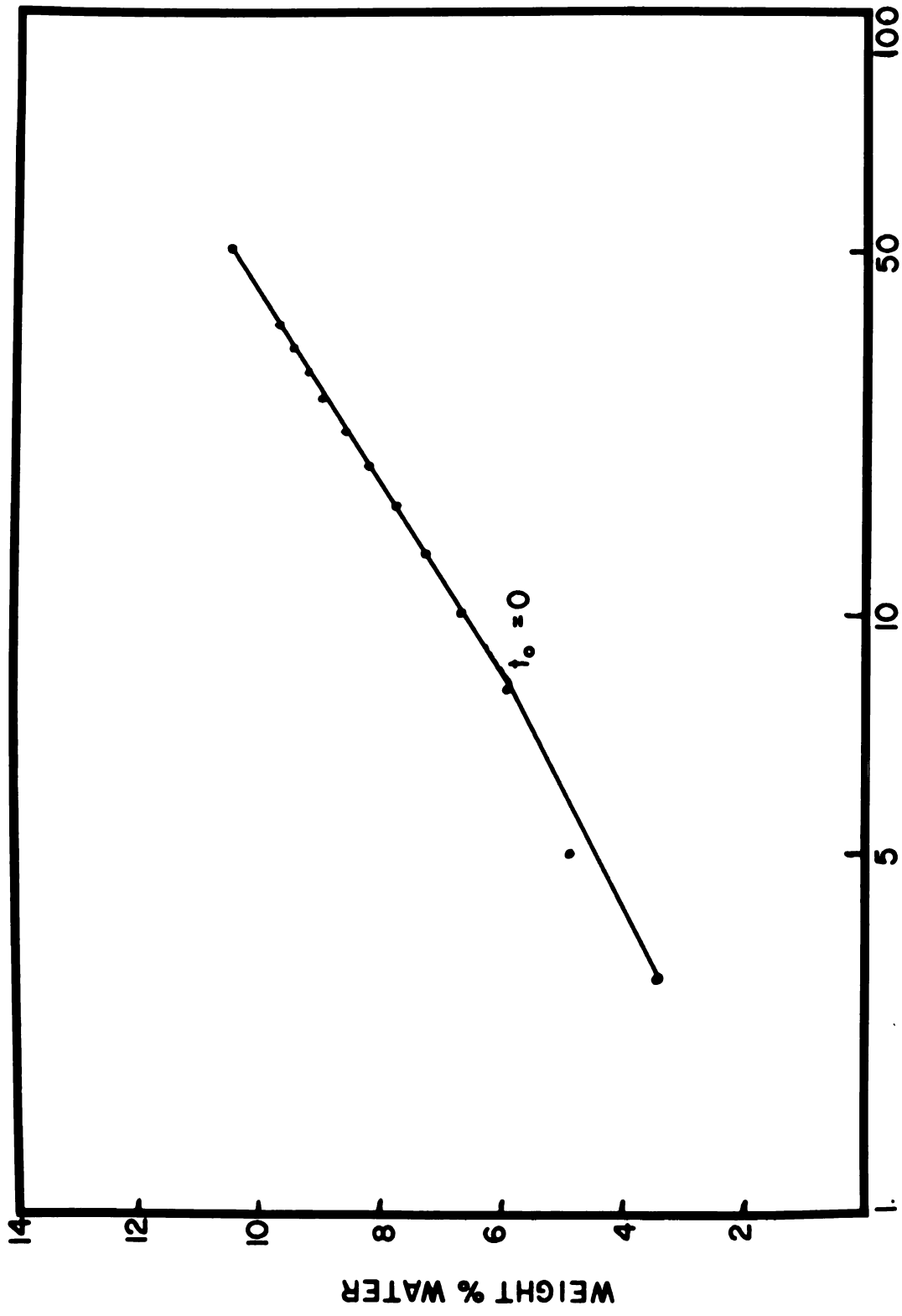


Figure 16. Adsorption kinetics of water on melanin.

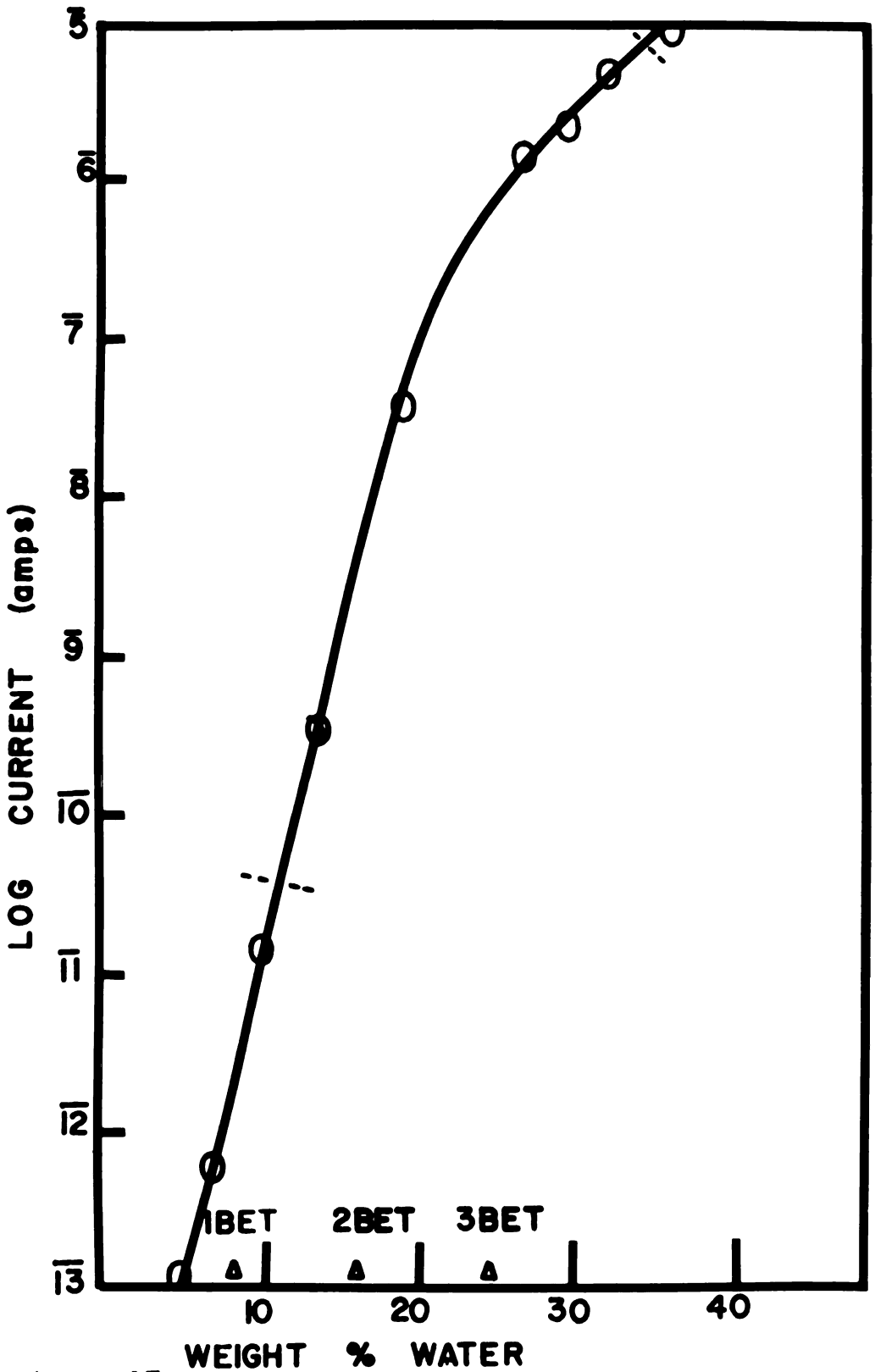


Figure 17. Conductivity of collagen as a function of adsorbed water.

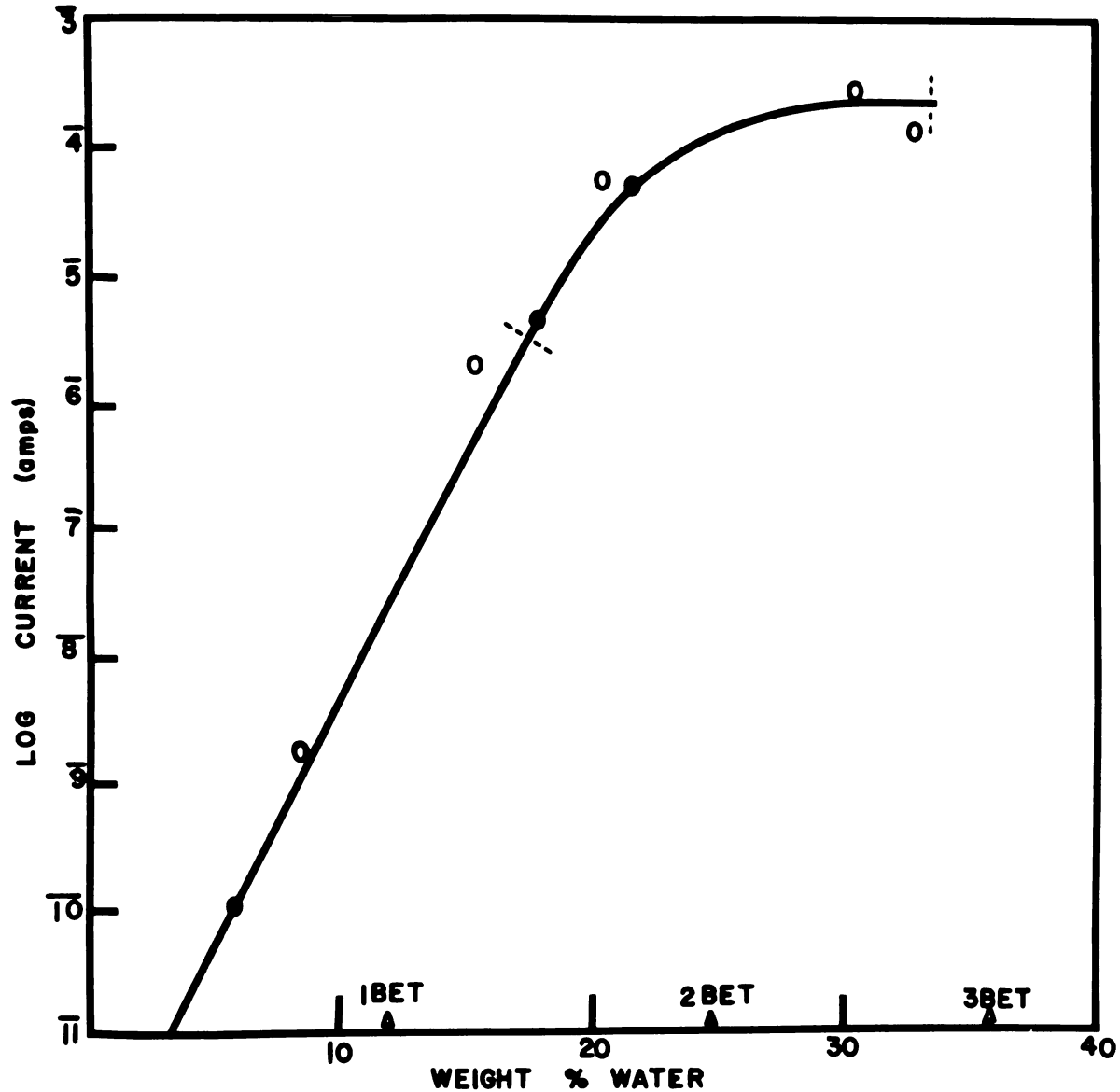


Figure 18. Conductivity of melanin as a function of adsorbed water.

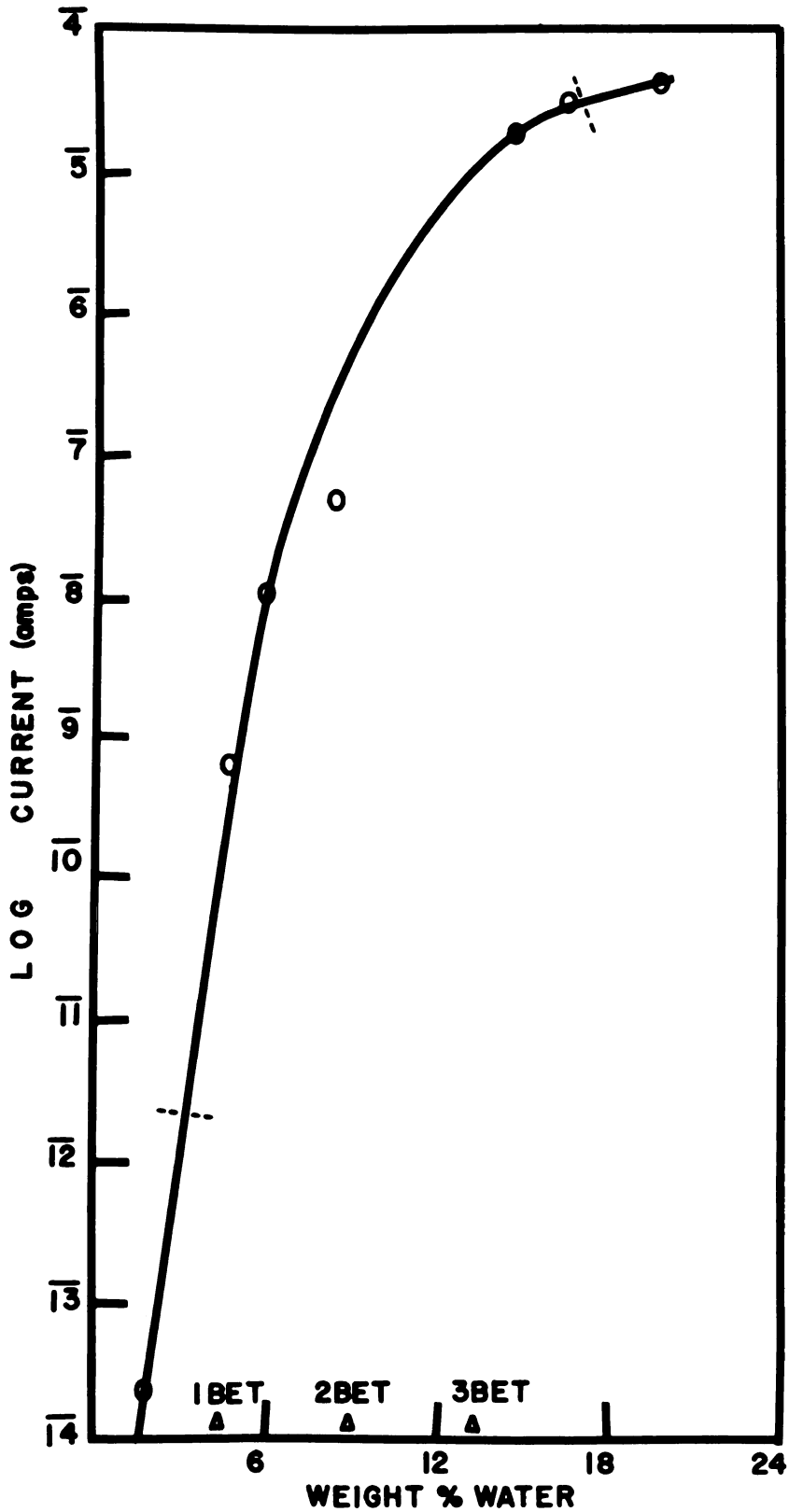


Figure 19. Conductivity of lecithin as a function of adsorbed water.

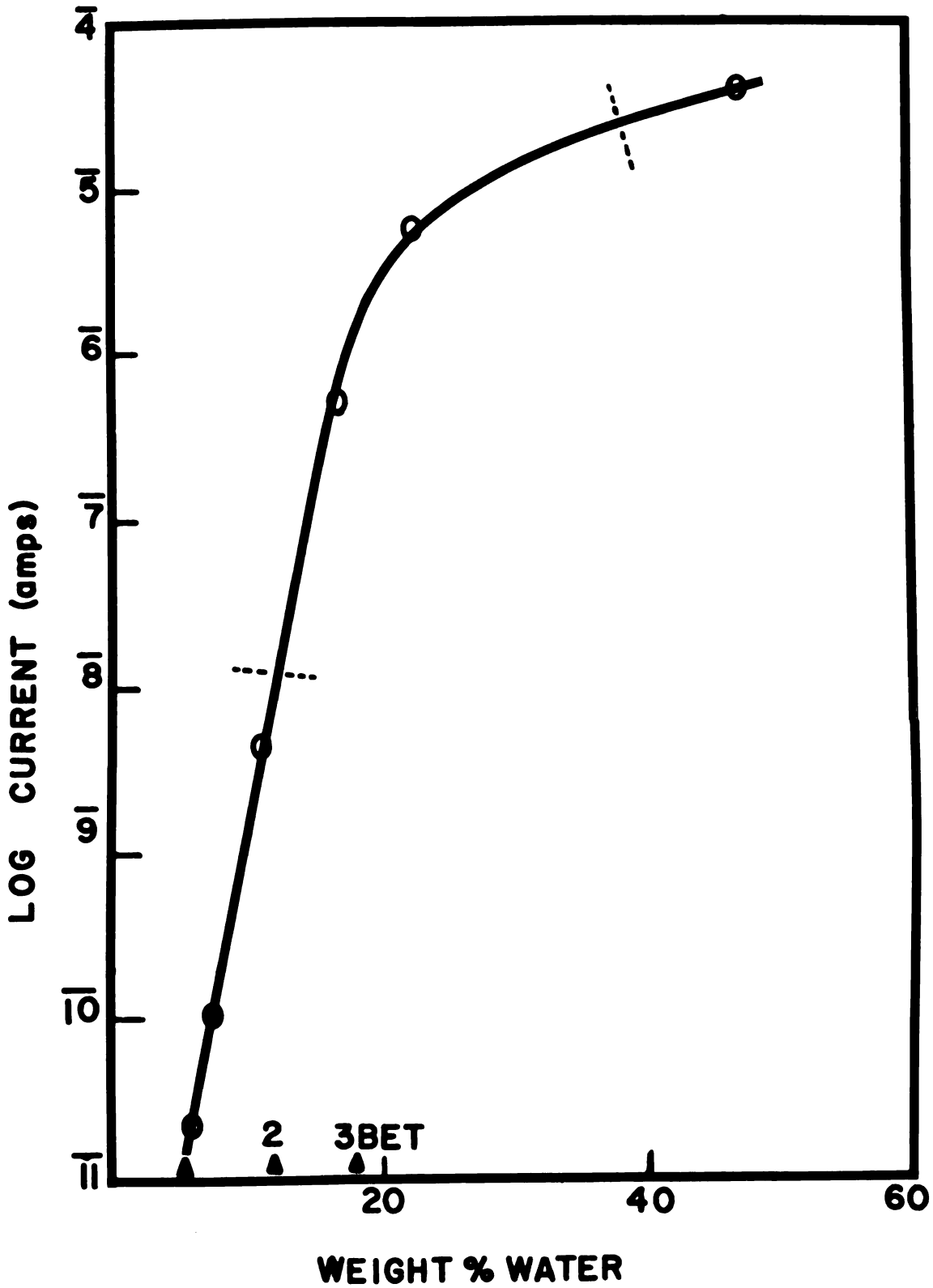


Figure 20. Conductivity of cytochrome-c as a function of adsorbed water.

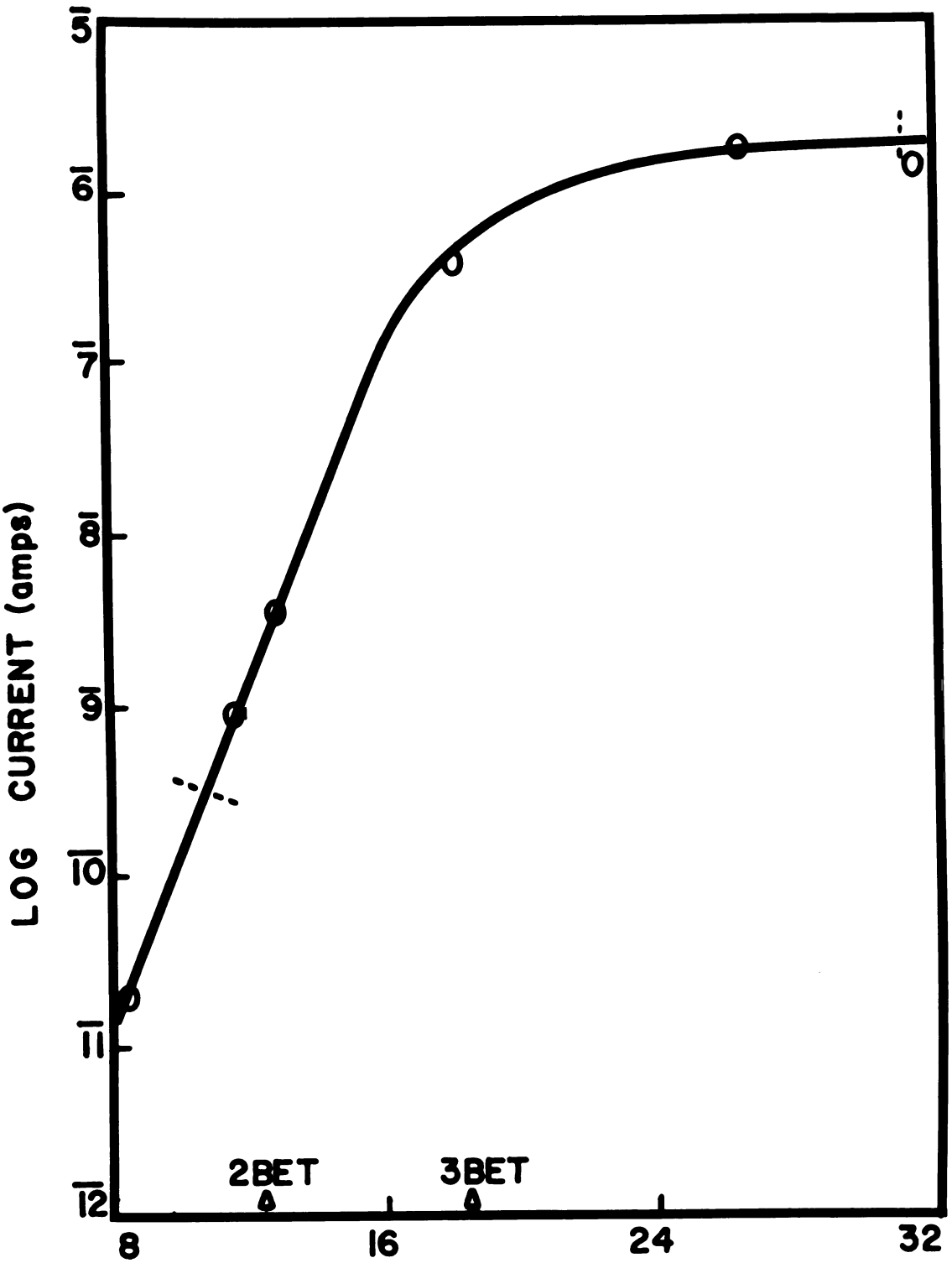


Figure 21. The conductivity of hemoglobin as a function of adsorbed water.

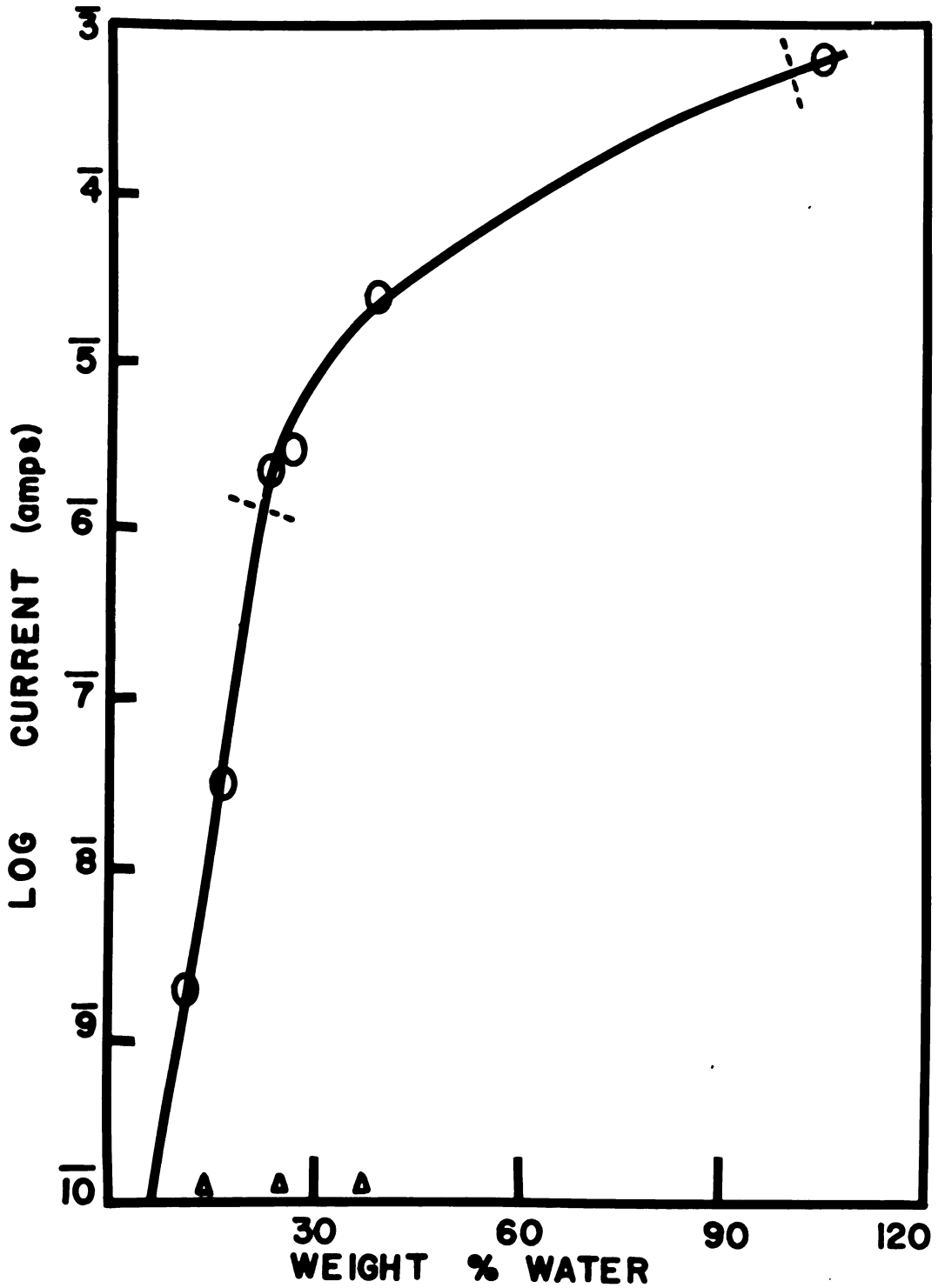


Figure 22. The conductivity of DNA as a function of adsorbed water.

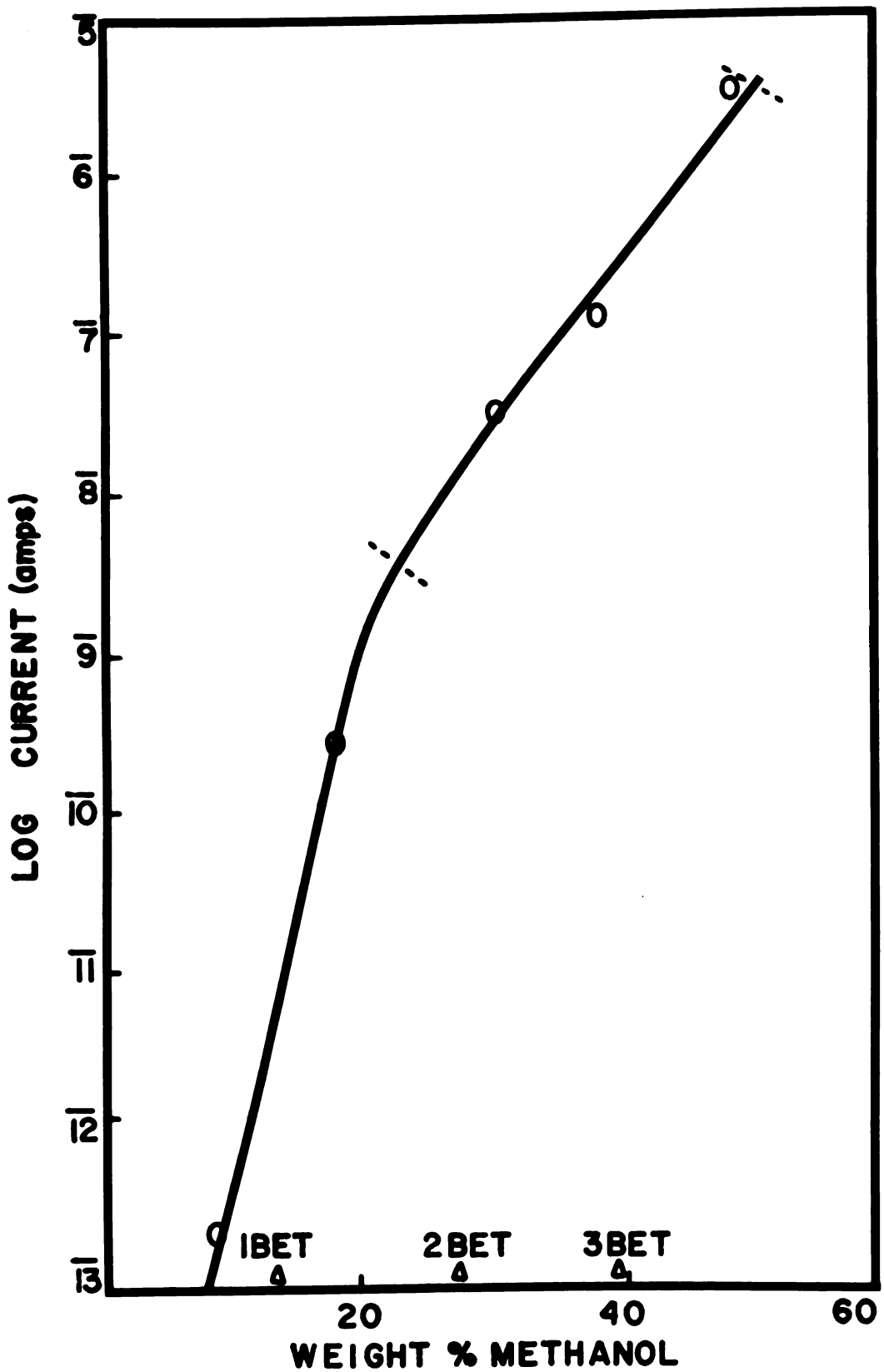


Figure 23. The conductivity of hemoglobin as a function of adsorbed methanol.

predicted by the Dielectric Constant theory as described earlier.

The curve in Figure 23 (hemoglobin+methanol) is calculated from an extrapolation of the Bradley adsorption isotherm for this system. Direct measurement of the entire range of p/p_o is not possible (only measurements to $p/p_o = 0.75$ have been done) with the present equipment as the Cahn Microbalance experiences problems in the presence of large amounts of methanol vapor.

Resistance - Voltage Measurements

It can be seen from the data in Figures 24 to 30 that Ohm's law is not strictly followed at low applied voltages. This is indicated by the non-linearity of the data for field strengths of less than 300 volts/cm. (30 volts applied). Above this value, the results indicate an almost ohmic behavior; this is characterized by an almost horizontal line. For low states of hydration, the samples displayed the Evershed effect discussed in the Introduction. Hemoglobin was the only compound examined which showed this effect over the full range of solvation. The various states of solvation for all the compounds are given as per cent solvations by the small numbers at the right side of the figure.

The possible variations from Ohm's law at the higher hydrations may not be real but only the result of obtaining incorrect measurements of the conductance in the presence of very large polarization effects.

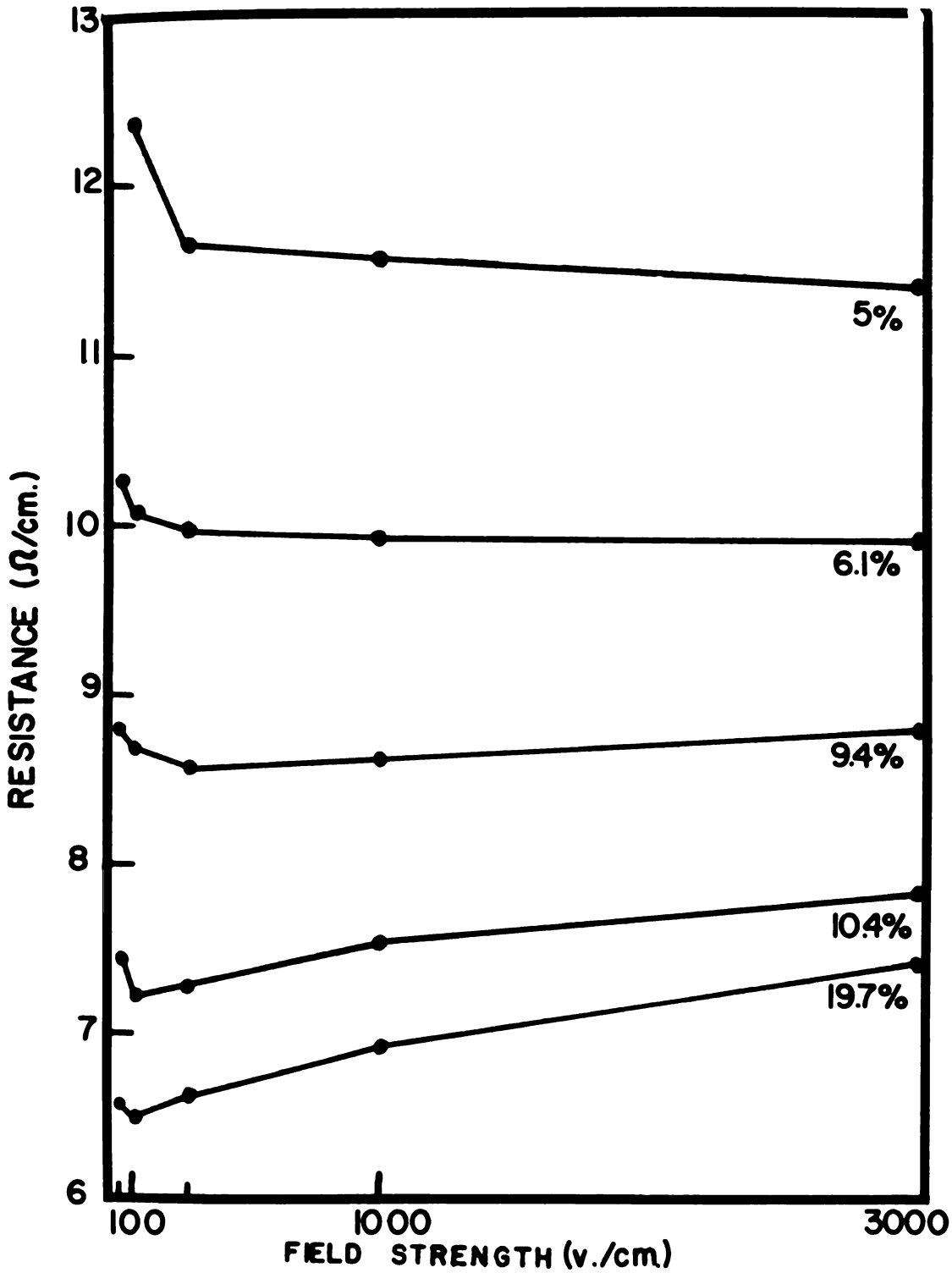


Figure 24. Resistance-voltage plots for hydrated lecithin.

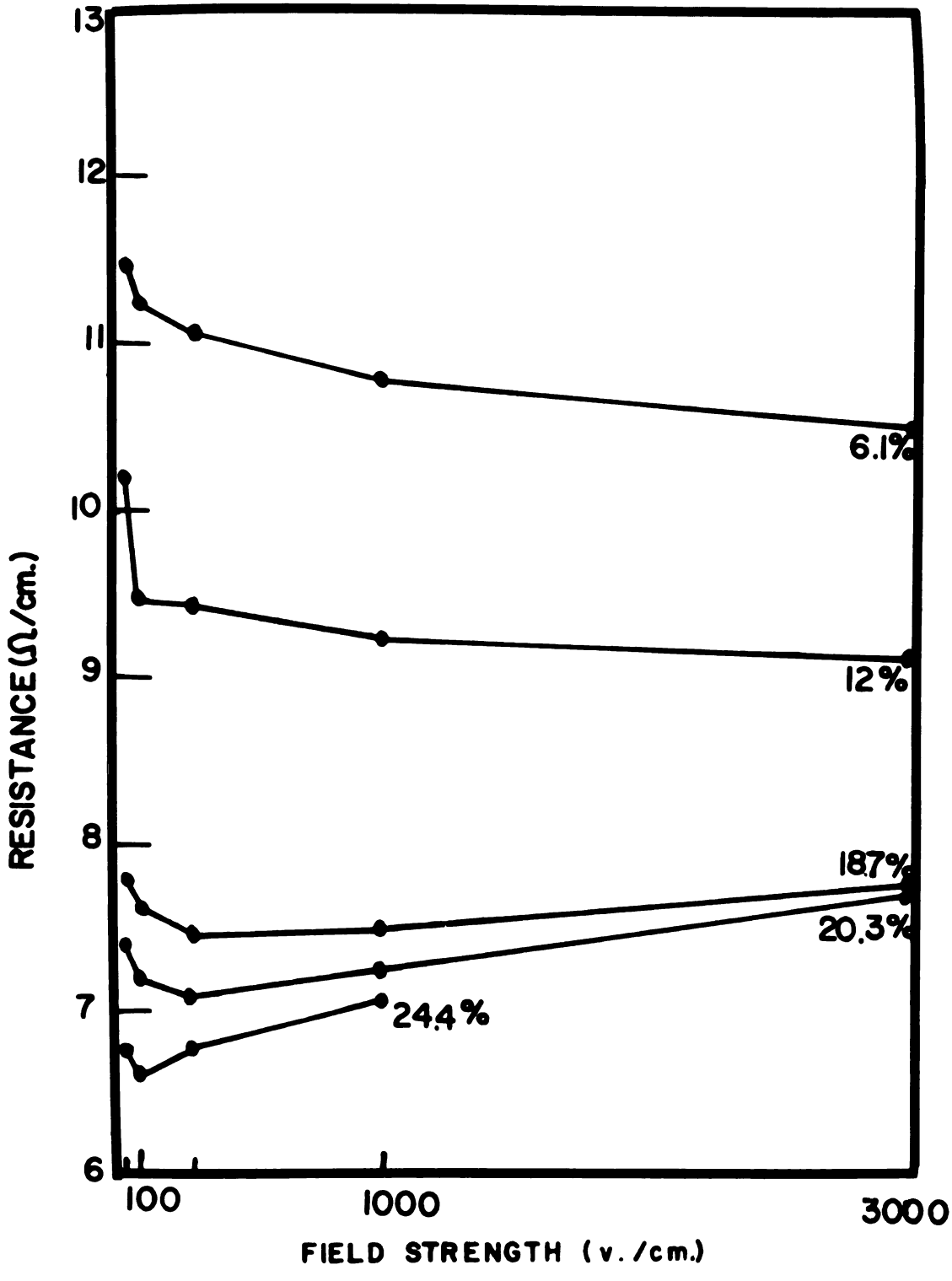


Figure 25. Resistance-voltage plots for hydrated melanin.

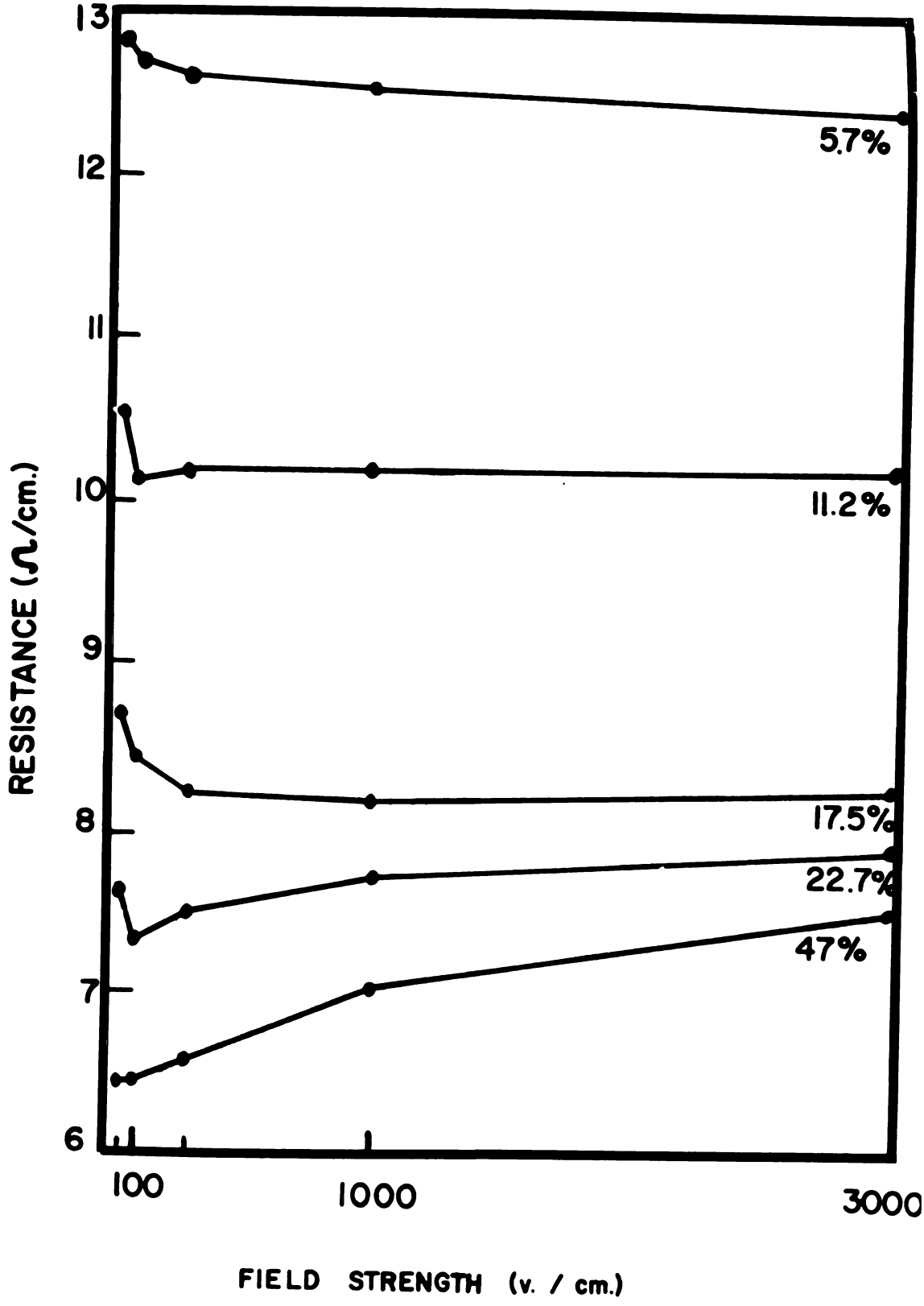


Figure 26. Resistance-voltage plots for hydrated cytochrome-c.

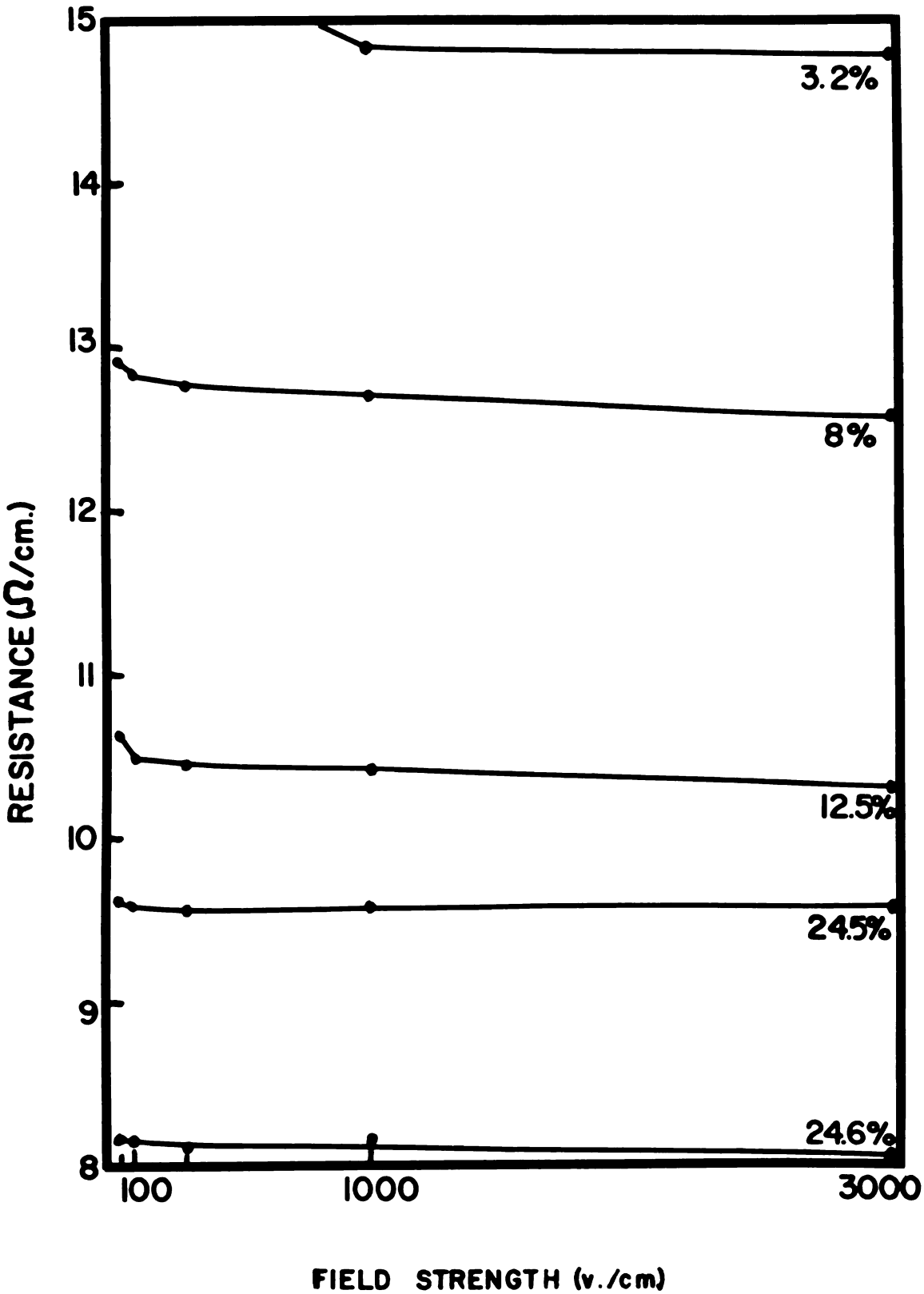


Figure 27. Resistance-voltage plots for hydrated hemoglobin.

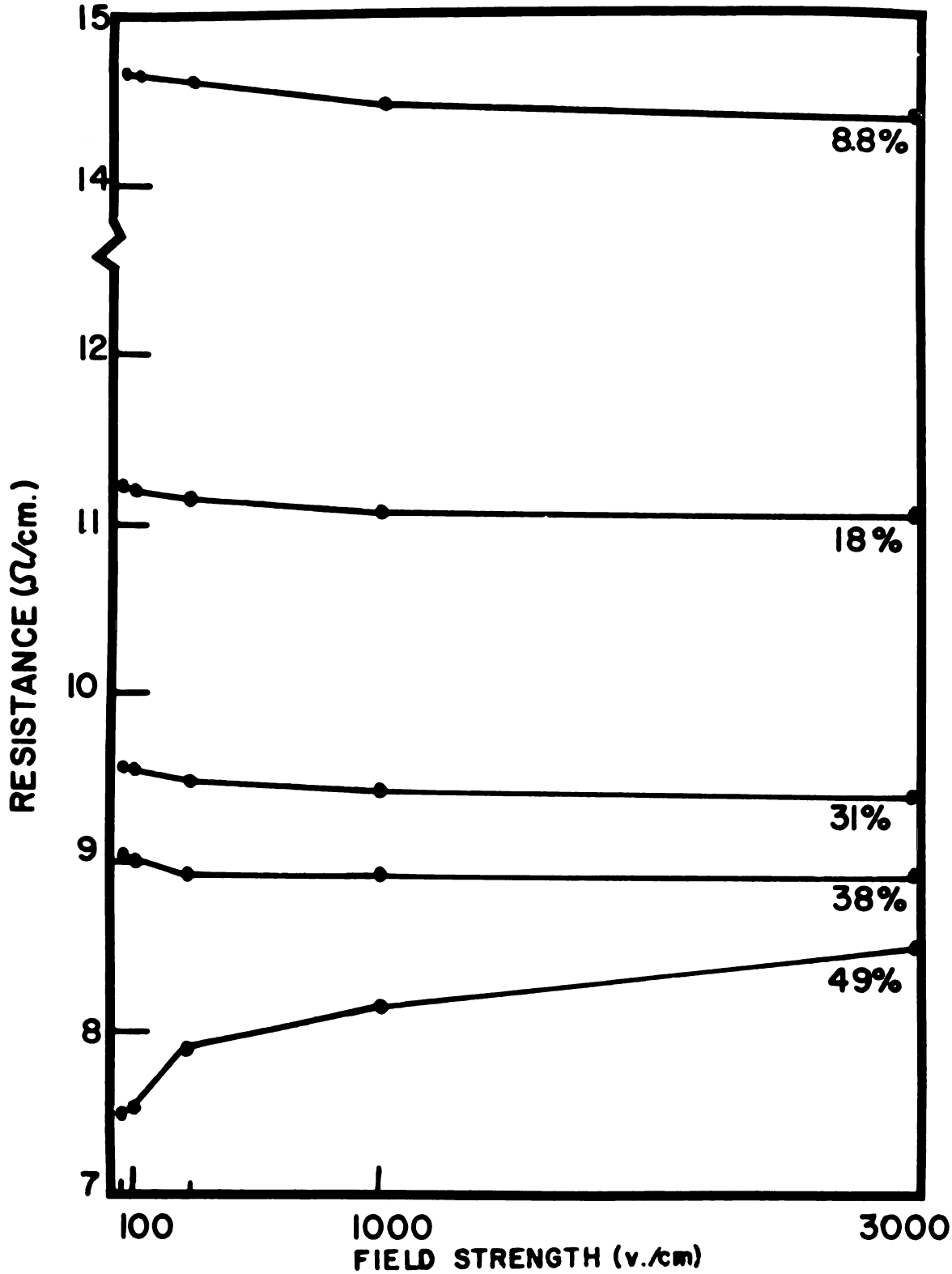


Figure 28. Resistance-voltage plots for methanol and hemoglobin.

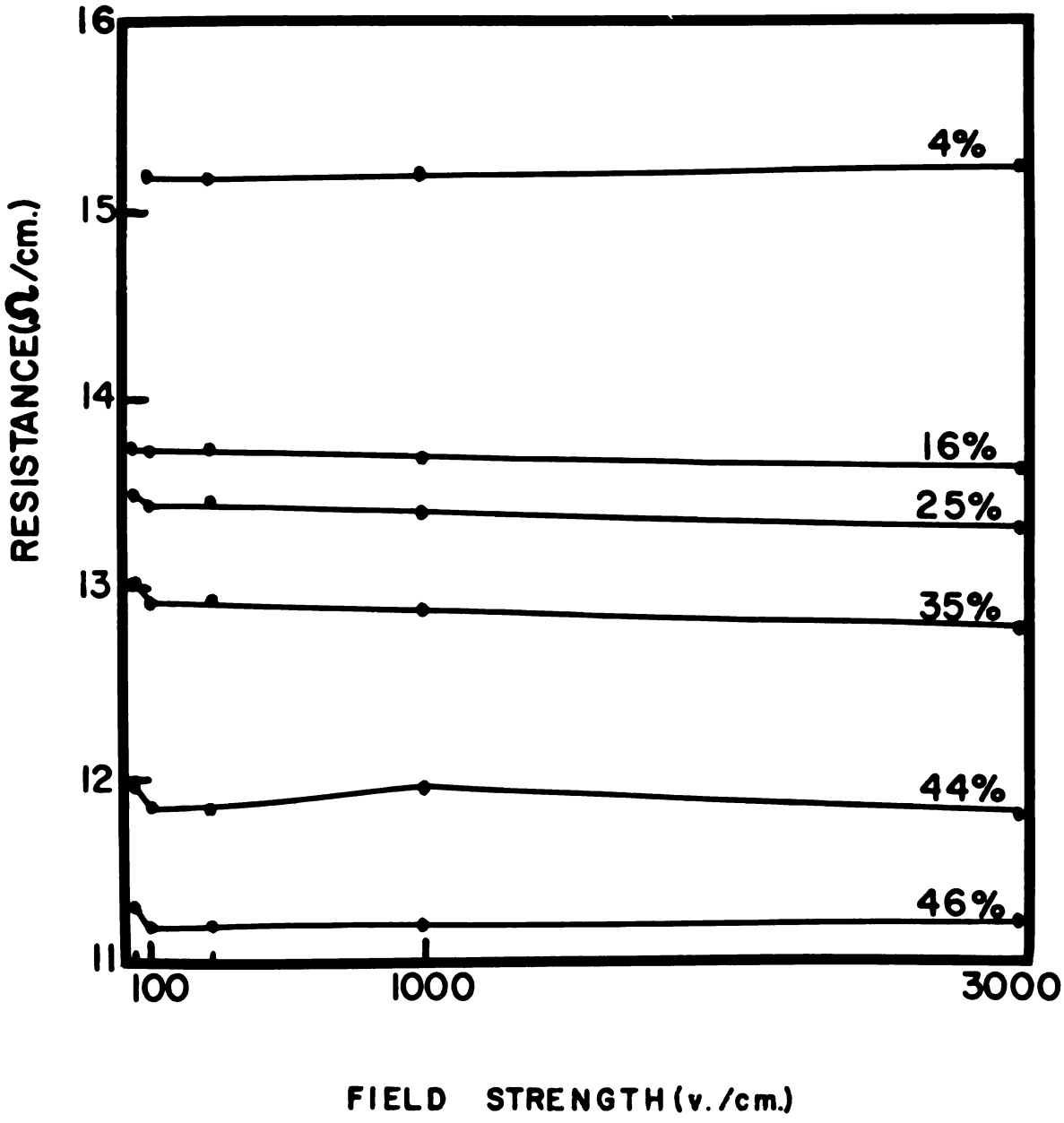


Figure 29. Resistance-voltage plots for ethanol and hemoglobin.

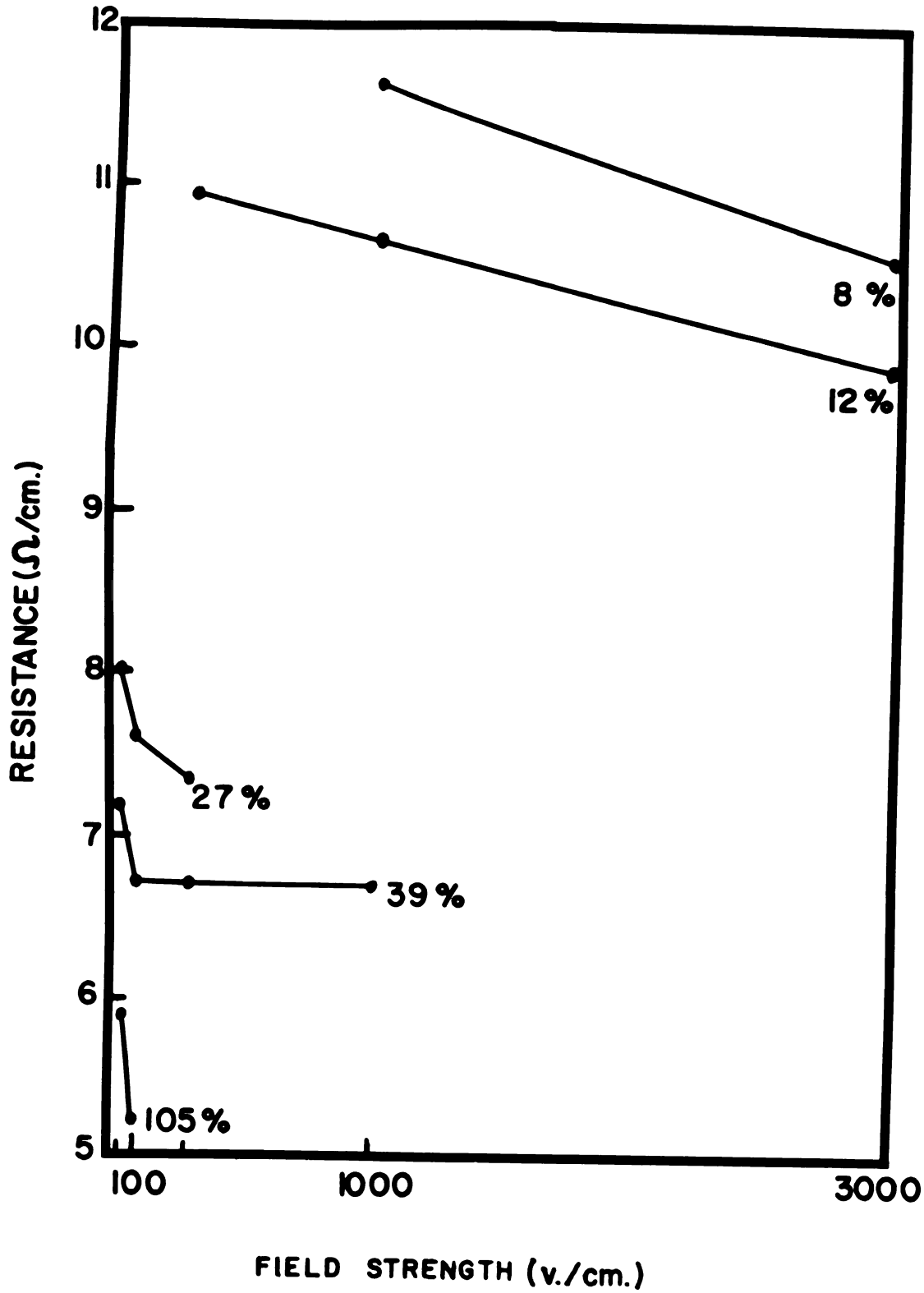


Figure 30. Resistance-voltage plots for hydrated DNA.

There appears to be no correlation between the line shapes of the Voltage-Resistance curves and the amount of electronic or protonic conduction.

Dielectric Studies

For the three materials studied, all produced curves of capacitance vs. hydration which showed a rapid rise after a hydration of about two BET monolayers. This is shown in the Figures 31 to 33.

Frequencies lower than 10^3 Hz. were not measured as it was observed by Postow (1968) that the capacitance had a large temperature coefficient in these ranges. In that a temperature coefficient is observed only in the term exp (-E/ 2kT) of the semiconduction equation, that is, the "activation energy" for charge carrier generation, the low frequency measurements do not apply to this theory. The increase of capacitance at low frequencies has been ascribed to Maxwell-Wagner polarization. This is produced by inequalities in the ratio of the dielectric constant and conductivities of the surfaces and the bulk volumes of the sample, or different components of the sample - water and protein, for example.

The dissipation factors of the samples measured were all characterized by a decrease in value with increase in frequency. This is at variance with the results found by other workers (Rosen, 1963; Brausse et al., 1968; Takashima and Schwan, 1965) but has been found consistently in this laboratory.

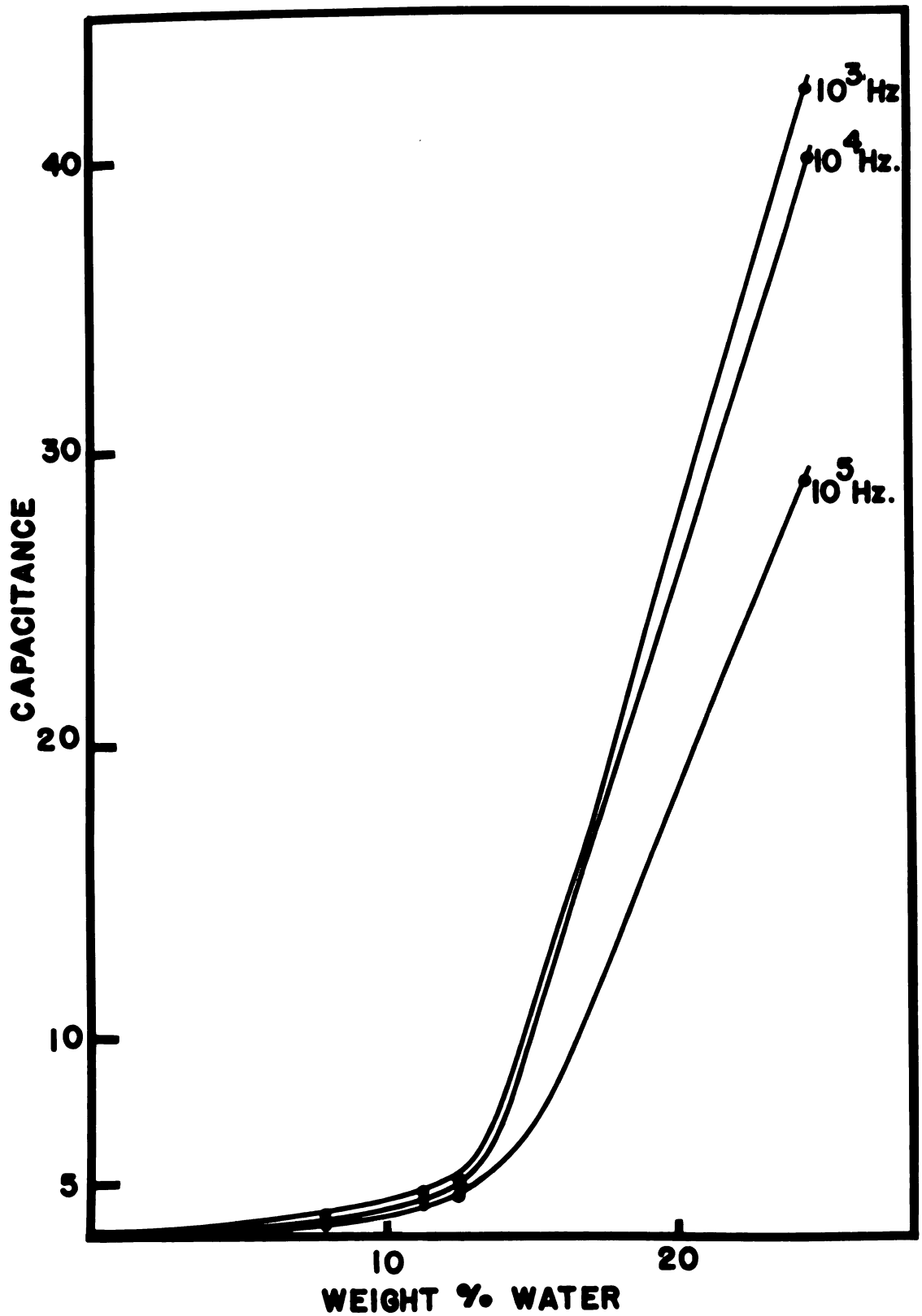


Figure 31. Capacitance vs. hydration for hemoglobin.

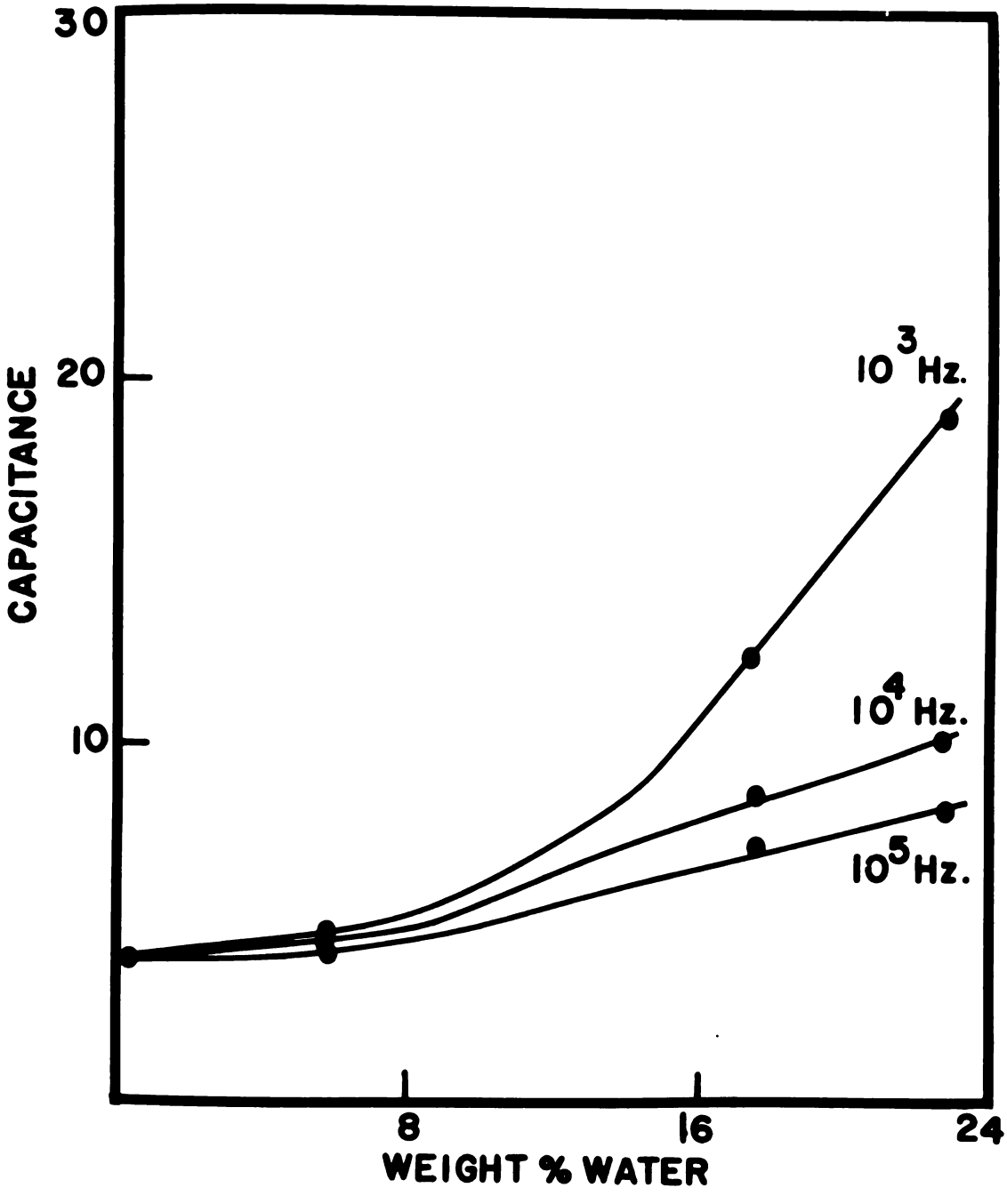


Figure 32. Catacitance vs. hydration for cytochrome-c.

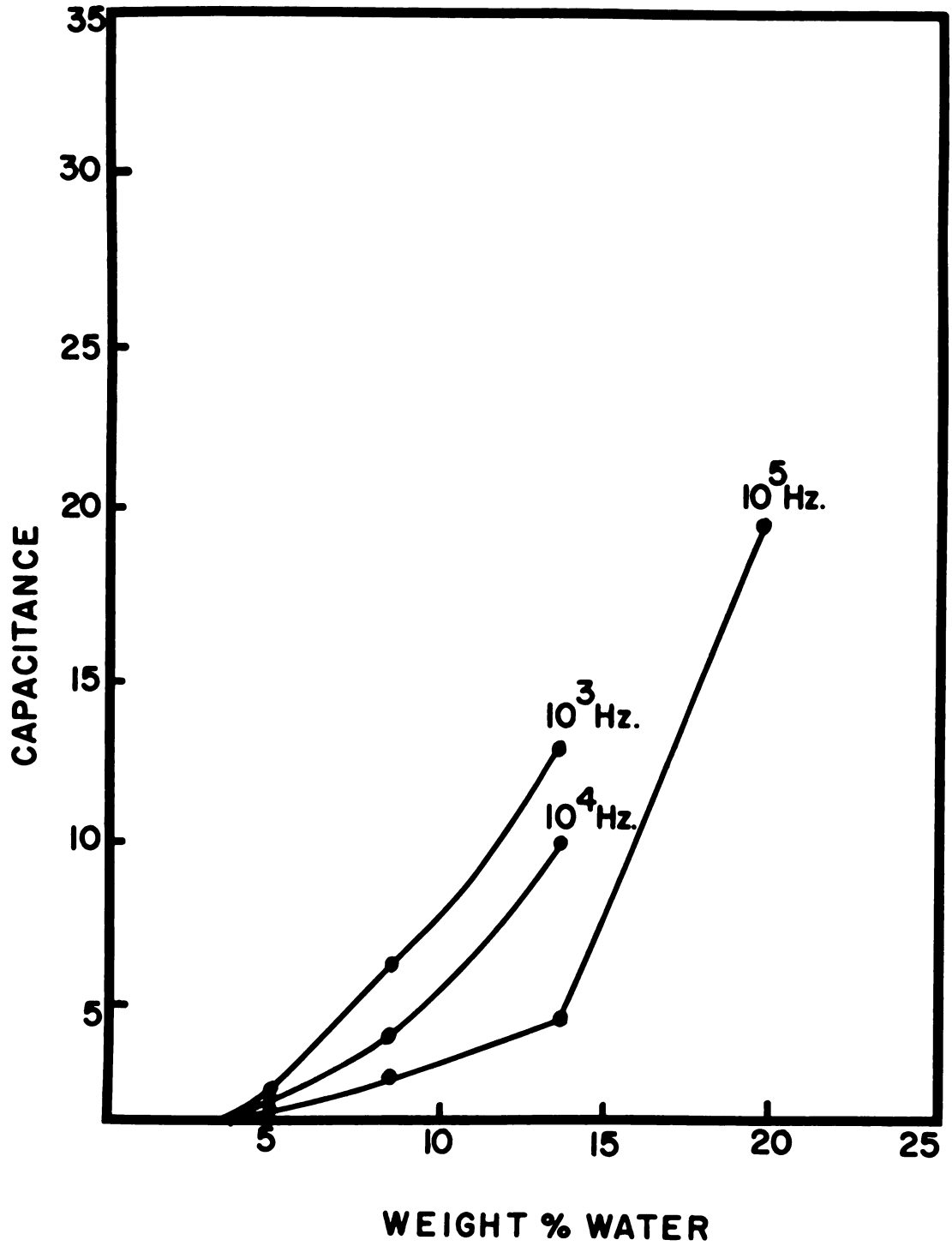


Figure 33. Capacitance vs. hydration for lecithin.

Electrolysis Experiments

The results for the electrolysis of compounds with varying amounts of adsorbed water are shown in Figures 34 to 40. The results are as expected, that is, increasing hydration results in increased protonic conduction. This is true for all samples with the exception of collagen, a triple helix with a high degree of hydrogen bonding.

The error bars on the graphs represent the 15% variation found in the electrolysis of oxalic acid dihydrate and appear to be quite realistic in estimating the precision of the measurements; small figures are experiment numbers.

Collagen, as mentioned, appeared to start out as a protonic conductor and become more of an electronic conductor with hydration. The bound water and the hydrogen bonding may be the cause of this large protonic mode. Small changes in capacitance with increased hydration may then result in a small change which allows the increase of electronic conduction. It must be remembered that the total amount of protonic conduction does not decrease, only the ratio of protonic to electronic carriers.

Melanin exhibited no change in the ratio of charge carriers through the entire hydration range of one to three BET monolayers. It was found to be the only compound to show this constancy.

Lecithin was tested in hydration ranges between 0.8 and 3.5 BET monolayers. It exhibited a gradually increasing protonic component starting from an even ratio.

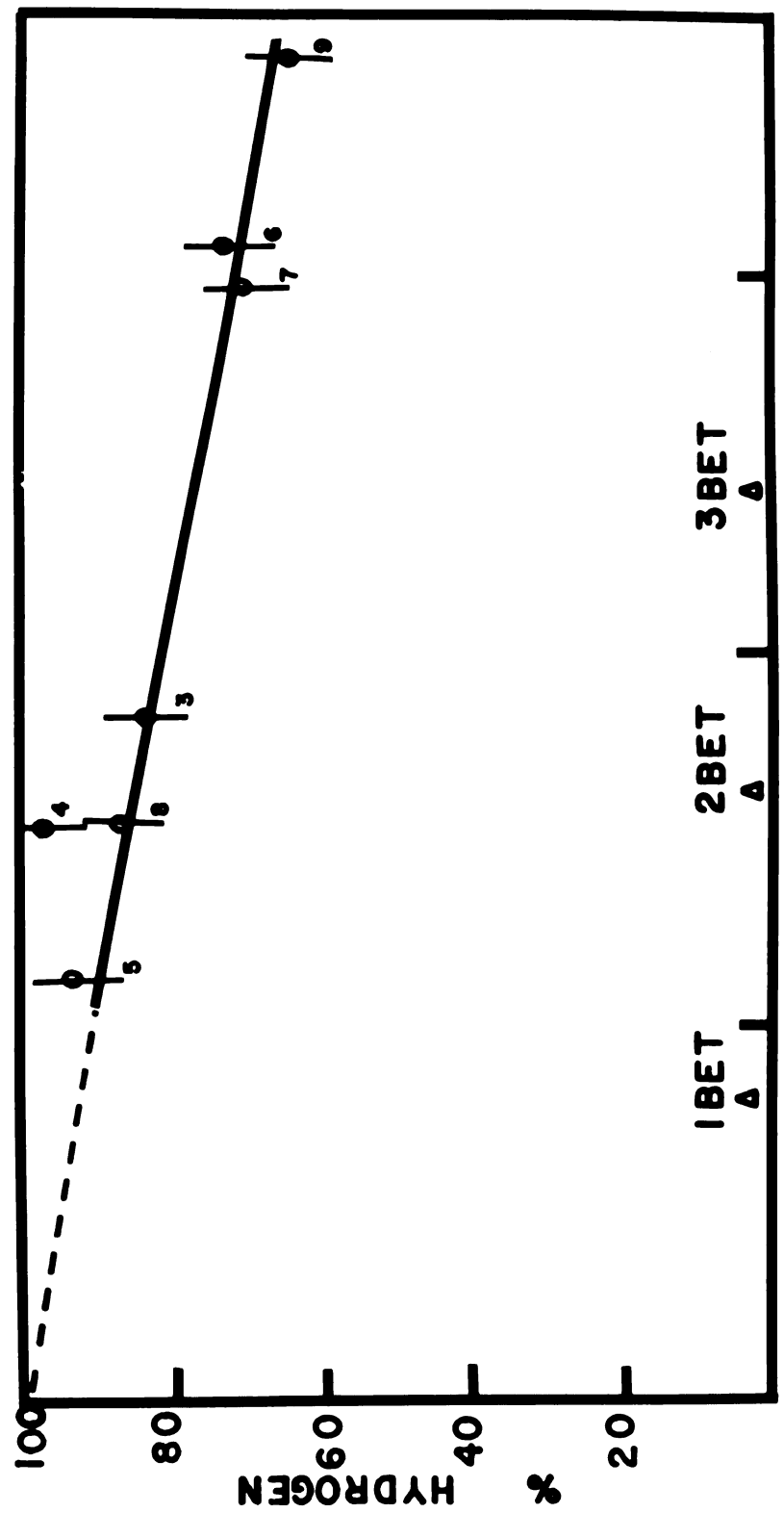


Figure 34. The yield of hydrogen as a function of hydration for collagen strips.

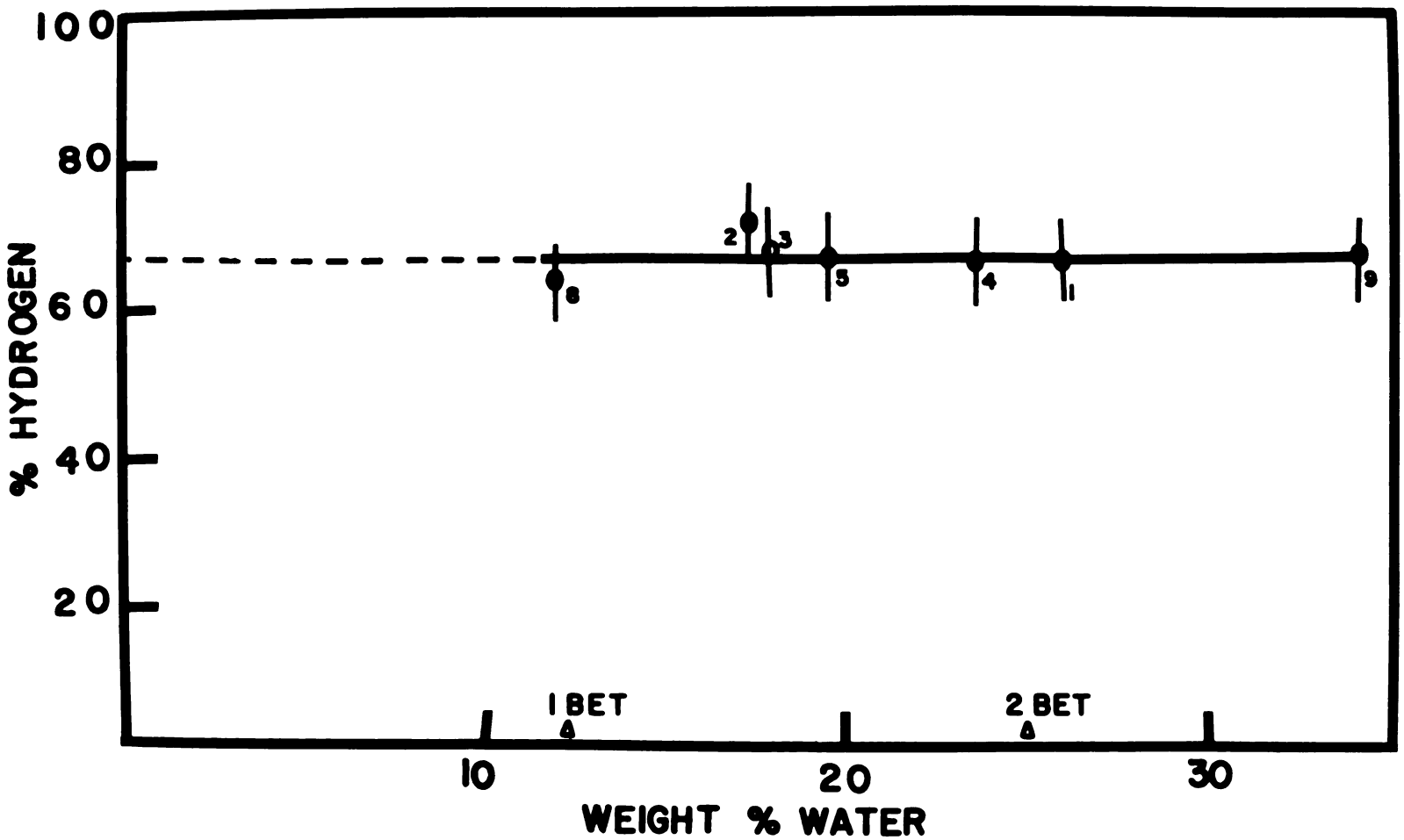


Figure 35. The yield of hydrogen as a function of hydration for melanin.

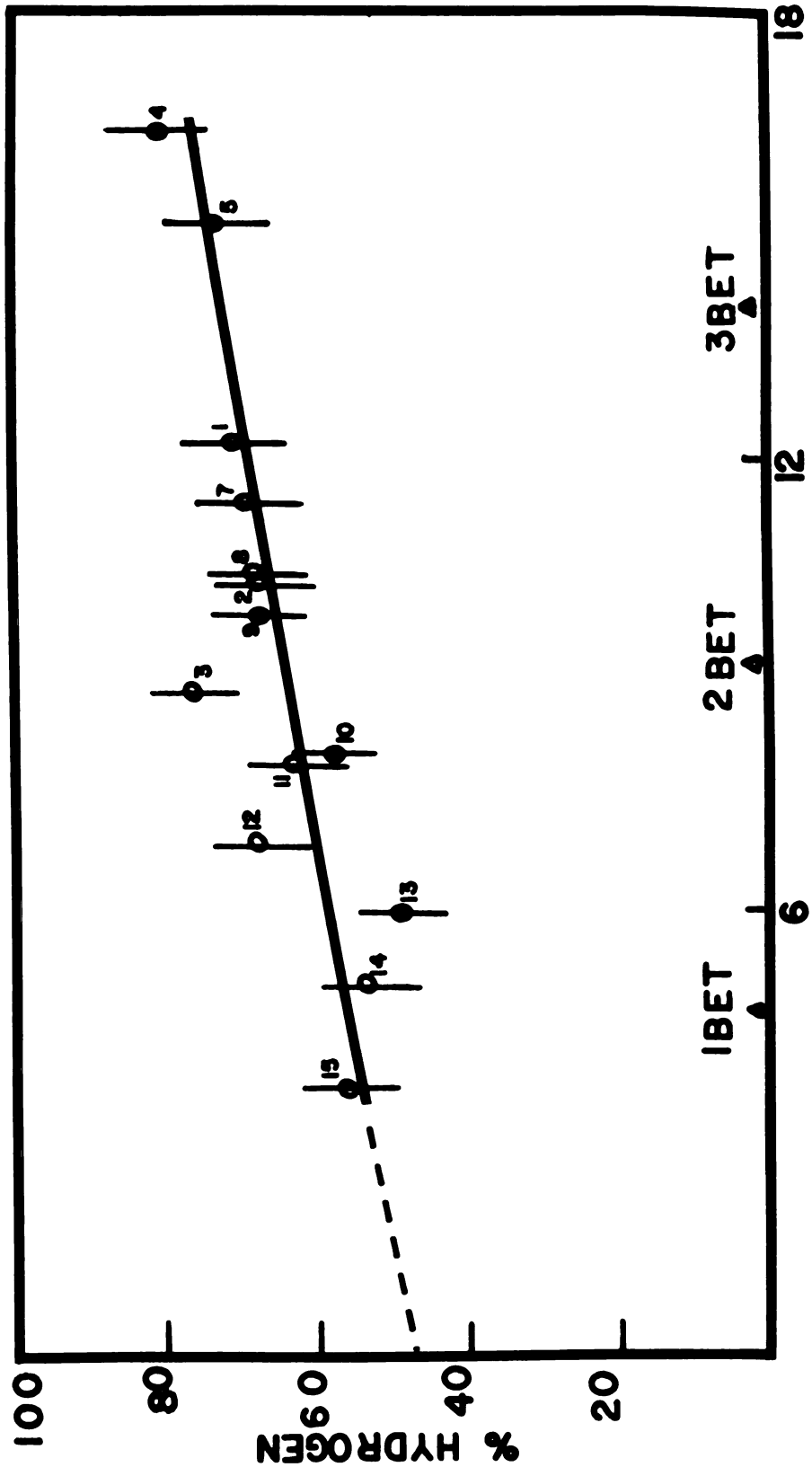


Figure 36. The yield of hydrogen as a function of hydration for lecithin.

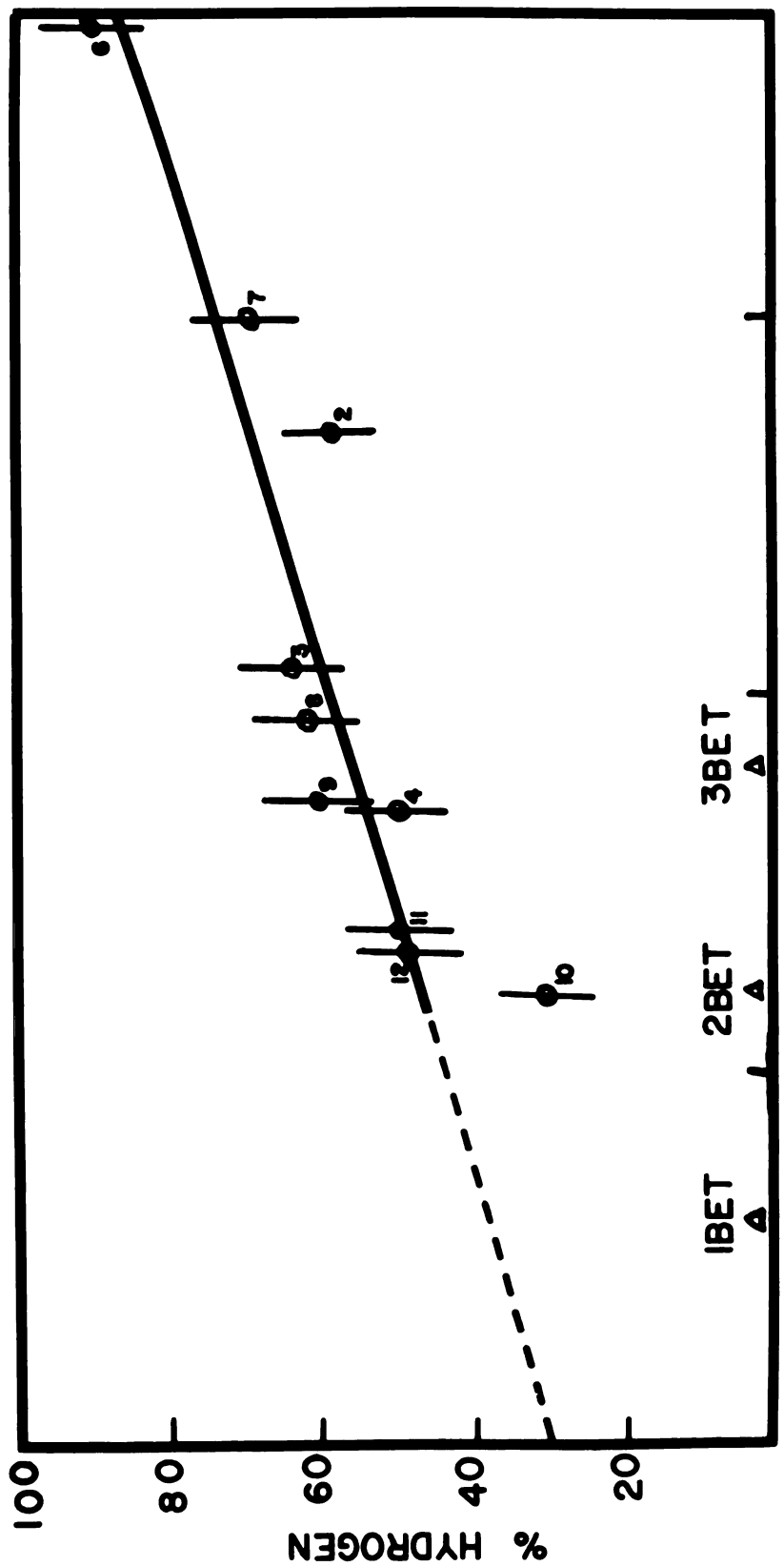


Figure 37. The yield of hydrogen as a function of hydration for cytochrome-c.

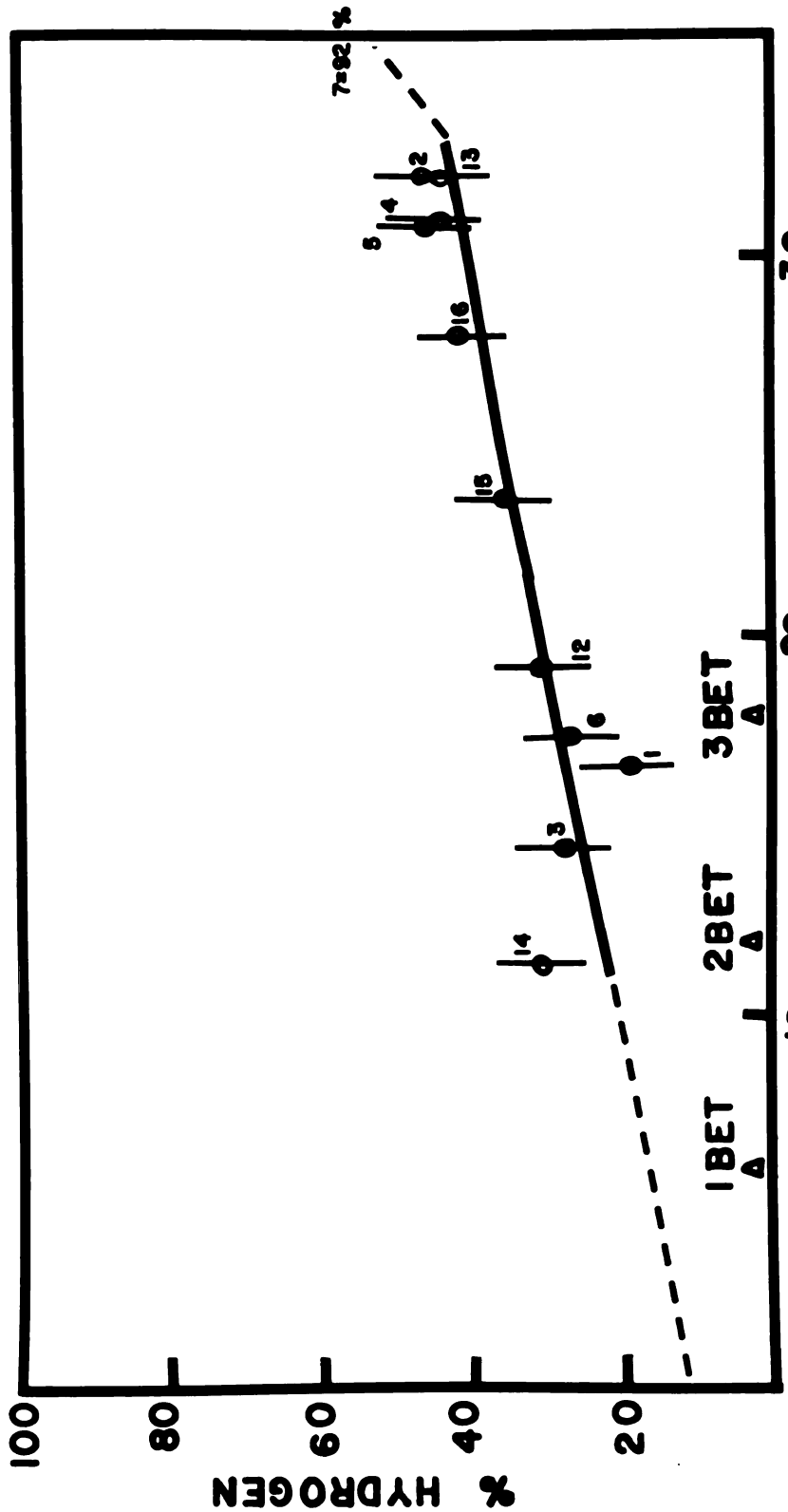


Figure 38. The yield of hydrogen as a function of hydration for hemoglobin.

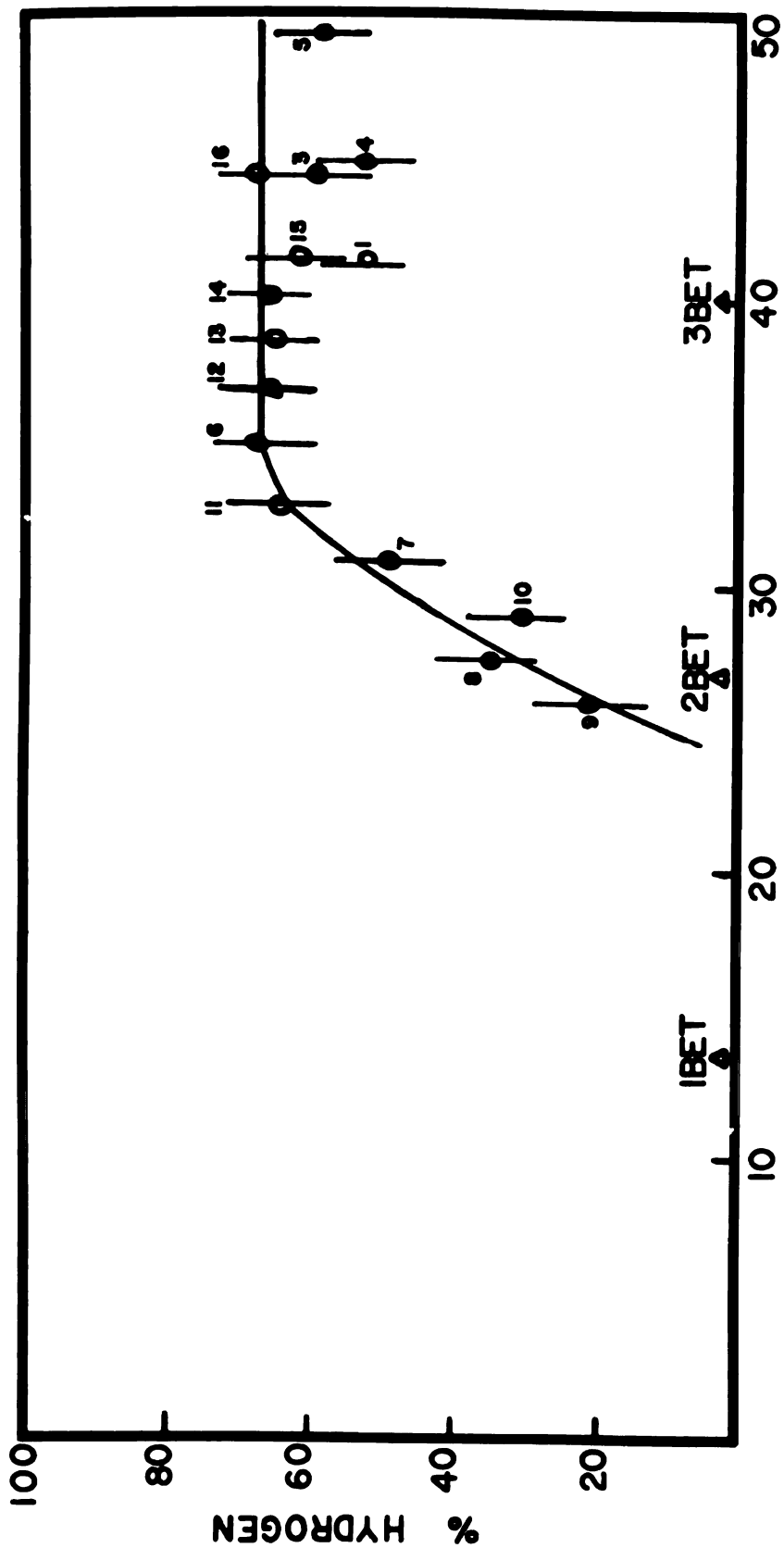


Figure 39. The yield of hydrogen as a function of solvation (methanol) for hemoglobin.

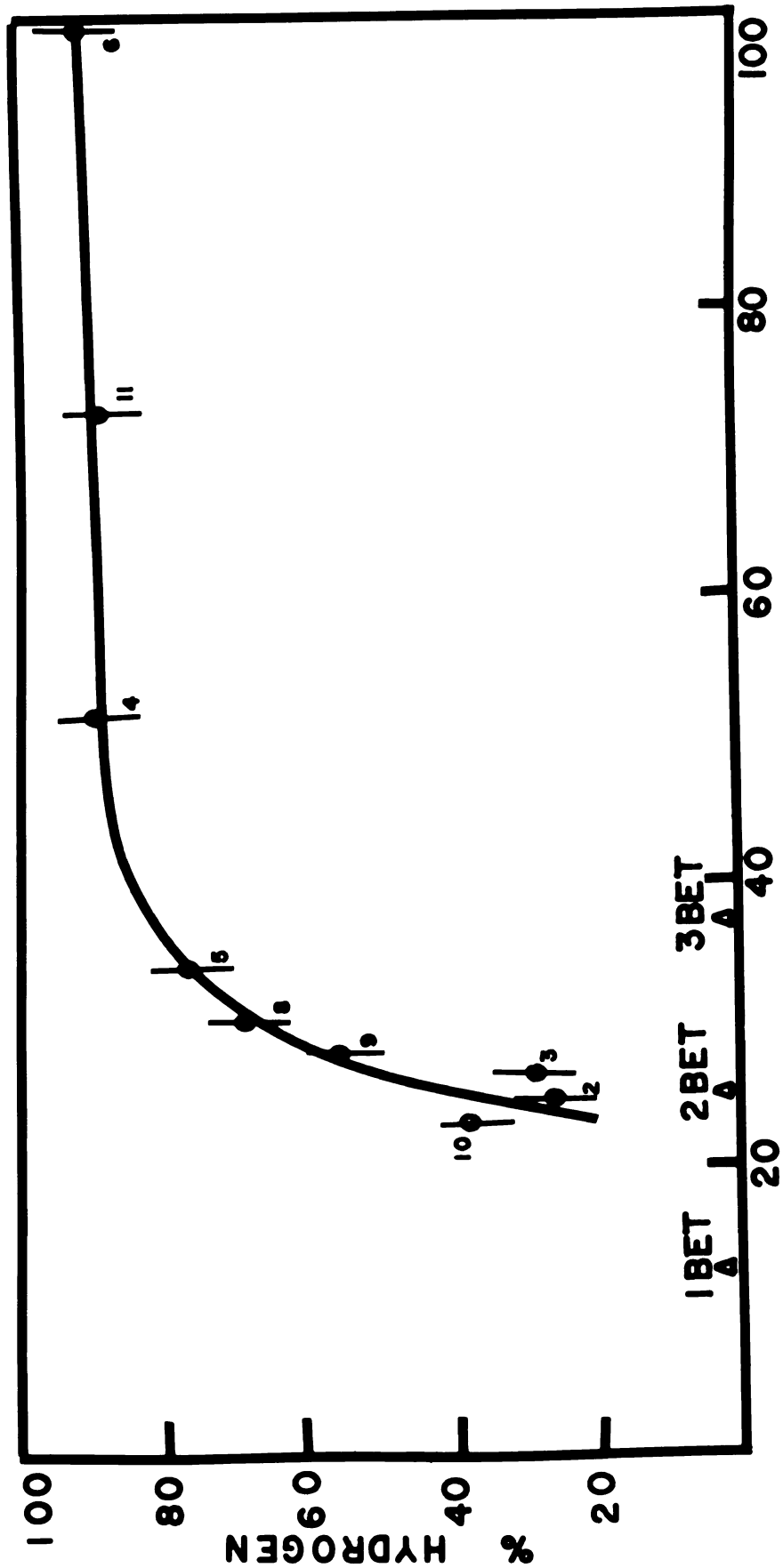


Figure 40. The yield of hydrogen as a function of hydration for DNA.

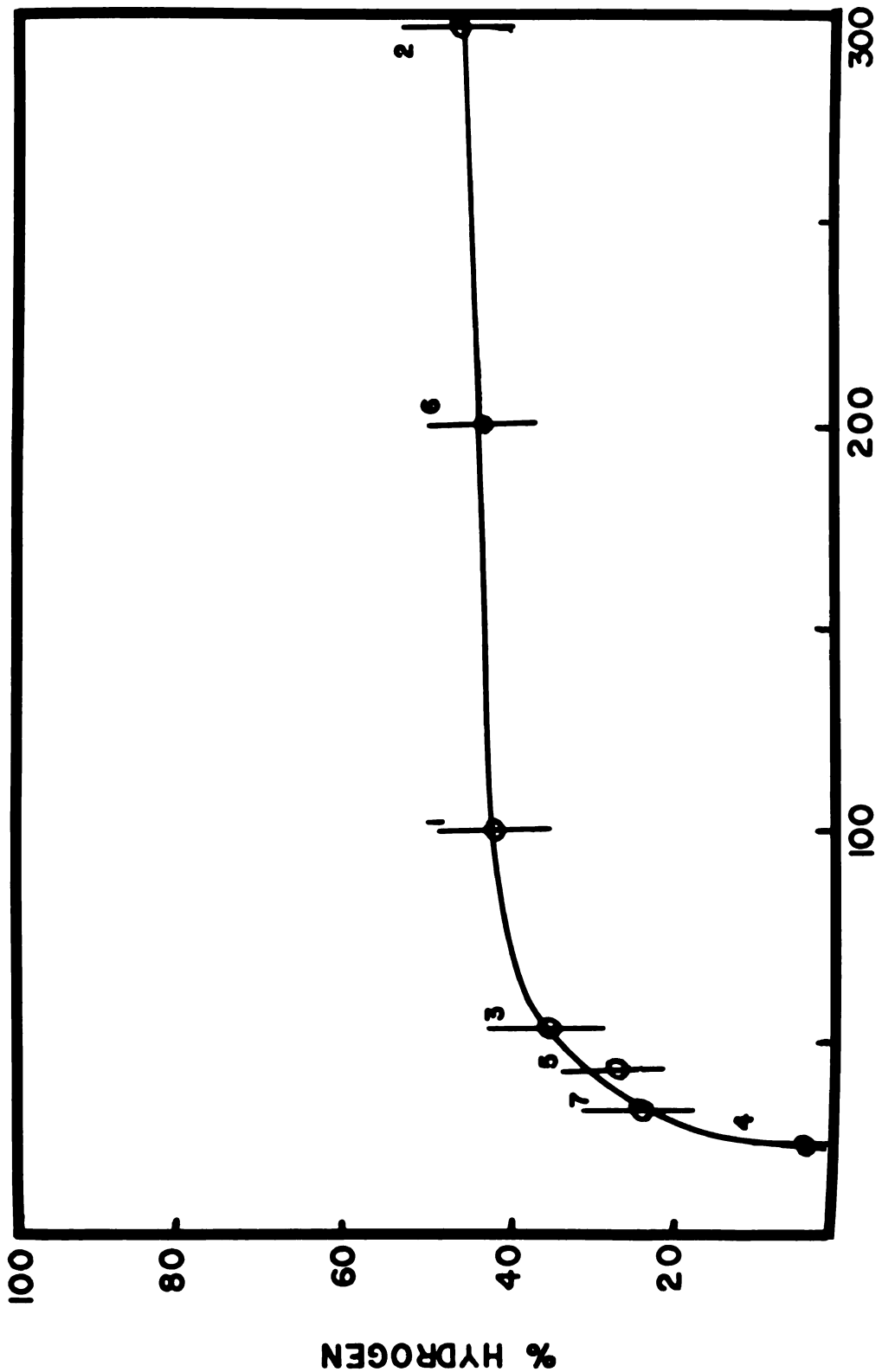


Figure 41. The yield of hydrogen as a function of voltage for one hydration state (28%) of hemoglobin.

Cytochrome-c was the only compound found to change from electronic conduction as the majority mode to protonic as the main form and yet vary linearly with hydration. The hydration varied from two BET monolayers to about six.

Hemoglobin exhibited a pattern of change which was very similar to cytochrome-c. This similarity was also seen in their adsorption isotherms and BET monolayers (6.1% for hemoglobin and 6.3% for cytochrome-c). An undefined hydration state for hemoglobin ($p/p_o = 0.98$), probably about 60%, gave a hydrogen yield of 92%; this is indicated at the side of the figure.

When hemoglobin was allowed to adsorb methanol instead of water, the change of carriers was not linear with solvation as shown in Figure 39. The cause for this is not yet known with certainty.

DNA showed a change similar to that for the methanol-hemoglobin system. Notice that the adsorbate was water and not methanol. This sharp change came in the range of two to three BET monolayers.

Tracer Experiments

Tritium exchange (tritium for protium) was measured for the two materials tested, hemoglobin and collagen, but only at one hydration state. Nevertheless, the figures for the exchange indicate an "order of magnitude" feeling for the process

For the hemoglobin system, the sample was studied at 8% to 10% hydration with the finding that the exchange was

about 54%. That is, of the initial activity of the water, 54% of it could now be found on the protein. (This should not be confused with the idea that 54% of the protons on the protein were now tritium.)

For the collagen system, one measurement was made at about 12% hydration; the exchange was about 38%. For these two systems, the hemoglobin was hydrated to 1½ BET monolayers, and the collagen was hydrated to 1½ BET monolayers.

The results of the electrolysis were unexpected in their small yield of tritium. The results are given in Table 2.

Table 2. The tritium yield for the electrolysis of collagen and hemoglobin.

Hydration State	Yield of Tritium
Hemoglobin:	
12.5%	2%
13.5%	3%
18.5%	1%
about 35 %	0.1%
" 35 %	0.5%
Collagen:	
13 %	4.3%
13 %	5.4%
very wet	0.23%

All values indicated a smaller protonic/electronic charge carrier ratio than given by the experiments measuring the change in pressure upon the evolution of hydrogen. This discrepancy is, at present, unaccounted for.

V. DISCUSSION

Biological Systems and Semiconductivity

It might now be asked, "What systems have been postulated in the past to involve the movement of electrons by semiconduction?". There are numerous examples, a few of which will be discussed here although it must be remembered that none have been proven.

Enzyme systems are among the most interesting possibilities for this semiconduction process. The activation energies (now figured on a 1/kT basis)are often of the order of magnitude of 0.5 to 1.0 e.v. (12 to 23 kcal./mole) which is the value for the semiconduction activation energy for hydrated proteins. The idea is theoretically appealing but is, unfortunately, difficult to prove on a system as small as one molecule - semiconduction is, at present, a macroscopic phenomenon. Additionally there are many theories of catalysis, all with their specific "proofs", and it would be difficult to relate many theories with one, simple mechanism. However, oxidation-reduction enzymes are a likely starting point.

LuValle and Goddard (1948) published a theory which could be considered as the forerunner of a semiconduction mechanism. Semiconduction was not known to them, but rather they used resonance over unsaturated bonds. These were

postulated to exist between electron donor and acceptor sites on different parts of the enzyme molecule.

Weber (1955) proposed a semiconduction mechanism for catalysis but later rejected it since the activation energy for charge carrier generation was larger than that for catalysis. He however used the dry state activation energy.

Cope has derived quantitative kinetic rate equations for oxidation-reduction enzymes. He however only uses the empirical fact that proteins are able to conduct an electronic current. The reaction rates are governed by diffusion and over-voltage and not by any semiconduction parameters. The current carrying ability is impressed, without modification, onto single-molecule systems.

In the realm of inorganic catalysis, Vol'kenstein (1963) has worked out a theory utilizing the semiconductive properties of many catalysts.

Rosenberg and Postow (1969) have called attention to the fact that changes in the molecular conformation of an enzyme could result in hydration changes and concomitant changes in activation energy. The activation energy change could then effect a change in the catalytic rate.

Such changes in activation energy at increased temperature was first noted by Crozier (1925). He postulated a series of reactions, one of which was rate-limiting, the "master reaction", and which governed the entire process. These effects were noted in biological systems. Among these were the chirping of crickets, the creeping of ants,

ciliary activity, the flashing of fireflies, oxygen consumption, bacterial growth, and the production of carbon dioxide. In many of these cases, there was an abrupt break in the Arrhenius plot, and this was frequently at about 15°C.

In vitro effects have also been observed involving a change in the activation energy, and Dixon and Webb (1958) have proposed the following explainations of the effect:

(i) a phase change in the solvent,

(ii) a change from one rate-limiting enzyme to another,

(iii) a parallel series of reactions, each with different activation energies,

(iv) reversible inactivation of the enzyme,

and (v) one enzyme, existing in two forms, with different activation energies for each.

It is this last explanation which is of interest to the theory of semiconducting enzymes. It is one however which is difficult to demonstrate with regard to semiconducting mechanisms.

From the time that Szent-Györgi proposed the idea of biological semiconduction, workers have been looking for instances to apply this fine idea. Arnold and Sherwood (1957) proposed that chloroplast grana were semiconductors, and, by this process, were able to transfer the light energy to the active, enzymatic site. The light was postulated to form electrons and holes which migrated separately to their respective "trapping centers" where they were utilized

in oxidations and reductions. Resonance transfer is more in vogue at this particular moment.

Arnold and Sherwood used the semiconductor model to explain their data on thermoluminescence. Bradley and Calvin (1955) proposed that, if the chlorophyll were to be positioned between lipoprotein layers, one of which is n-type and and the other of which is p-type, the photo-generated electrons would be able to diffuse to their respective sites of chemical activity.

ESR work at low temperature by Calvin and Androes (1962) and Calvin, Kurtz, and Ruby (1965) has given some support to the semiconductive hypothesis, but it has also been applicable to quantum-mechanical tunnelling. The hypothesis is that exciton migration brings the electron-hole pair to the enzymatic site; there triphosphopyridine nucleotide is oxidized and the hole migrates to another site where it either recombines with another electron which has been photoexcited, or it combines with an electron from oxidized cytochrome-c. These processes would occur in green plants and photosynthetic bacteria, respectively.

Eley and Snart (1965) have found that charge separation occurs in complexes of protein-chlorophyll-carotene-β-methyl napthoquinone. These complexes exhibited different semiconductive activation energies than the components.

The chemiosmotic hypothesis of Mitchell (1961) proposes that the link between metabolic energy and oxidative, or photosynthetic, phosphorylation is the membrane-

based, reversible ATPase. The membrane must be impermeable to protons and possess electron-transporting components. The nature of this conduction is not known, but it could be a semiconductive one.

E. J. Conway (1953) has suggested an ion transport mechanism based on the movement of electrons in the membrane components. These electrons, or negatively charge "carriers", would then combine with cations to form a neutral species capable of migration through the membrane. At the opposite side of the barrier, the electron would be removed, and the complex would dissociate thus releasing the ion.

Cytochrome compounds (cytochrome-b_5) have been detected in the membranes of endoplasmic reticulum (Remmer and Merker, 1965). These cytochromes have been implicated in ion pumps by Davies (1960) and also in functions such as the terminal oxidation reactions in the synthesis of proteins. These latter reactions would also involve the shuttling of electrons from NADH to various molecular acceptors. Additionally, biotransformation of pharmacological compounds takes place at the endoplasmic reticulum.

Again in the area of membranes and boundries, is the finding by Digby (1965) that the cuticle (quinone-tanned protein) of various crustacea is capable of conducting electrons. This was demonstrated by electrodeposition of copper on the surface of the cuticle. Digby has proposed that a cathodic reaction with the salt water (the animal is normally negative with respect to the water) results

in the formation of an alkaline pH in the area. This promotes the calcification of the animals exoskeleton.

Recent experiments by Rosenberg and Pant (1969) may indicate that membranes (bimolecular membranes of cholesterol-oxidized choloesterol) are electronic conductors at low potentials and ionic conductors at higher potentials. The research is in a preliminary stage at the time of this writing.

Rosenberg (1966) has demonstrated a similarity between the photoconductive and photovoltaic responses in model systems and those observed in the eye - this is particularly with respect to the S-potentials. Misra et al.(1968) have proposed a mechanism of olfaction based upon semiconduction.

Energy migration in the mitochondrion has long been an area of active interest as it is known that the electron transport chain is made up of enzymes which are rigidly fixed to the cristae. This means that electron transfer between the enzymes can not occur by diffusion. Movement by molecular rotation, in some parts of the chain, is not possible as reduction of cytochrome can take place at liquid helium temperatures as discussed earlier in this work. Semiconduction is one alternative.

Cope (1965) has developed a kinetic treatment of the electron transfer question and has tested it in vitro. For living systems, there is still some debate.

In the systems prepared by Chapman and Fast (1968), it was found that chlorophyll in aqueous glycolipid and phospholipid dispersions were able to reduce an aqueous

solution of cytochrome-c in the presence of light. This reaction will not take place in an ethanol solution of the components. The recent experiments of Tien (1968) have domonstrated charge separation in bimolecular lipid membranes (BLM) containing chlorophyll in the presence of light.

Future Improvements in the Electrolysis System

The primary question to which this study has been directed dealt with the nature of the charge carriers at various levels of solvation. For this purpose, various facets of the adsorption process have been studied, and the electrolysis experiments have been carried out. At no point in this sequence of experiments has it been possible to study the very dry macromolecules, and therefore the nature of the charge carriers in this region can only be inferred from extrapolation. From the theoretical point of view, this can be a dangerous proceedure. But from a position of relevance to the biological realm, it is only the solvated systems which are of importance for it is these alone which possess the necessary low resistance to make electron transport rates significant.. The penetration of the low solvation region is then the "new frontier" and awaits the development of a more sensitive device.

An attempt was made to improve the sensitivity of the equipment by replacing the McLeod gauge with an ionization gauge. This arrangement suffers from the problem of not being able to detect the hydrogen against the background

pressure of nitrogen, oxygen, and water, the principal gases released during the out-gassing of the walls of the vacuum chamber. Since the ionization gauge responds in a different manner for each species of gas (the current measured by the gauge controller is a function of the ionization potential of the gas), unless only one species of gas is present, an accurate determination of the pressure of that gas is not possible. Hydrogen would have to be the major component by far.

To separate the hydrogen from the other gaseous components of the system, a palladium tube was placed such that the hydrogen gas passed into a previously well-pumped ionization gauge instead of to the atmosphere. Unfortunately, the reaction of palladium and hydrogen (as yet not known) prevented all but a small fraction of the hydrogen to penetrate the palladium tube and enter the ionization gauge. This problem was also found by Young and Whetten (1960) for their hydrogen measuring device based on the palladium tube. It became necessary to generate such large amounts of hydrogen to compensate for this loss, that one did not achieve any more sensitivity over the sensitivity of the device used for this work. In addition, one no longer had an absolute gauge as possessed by utilizing the McLeod gauge. After five months of testing, this system was rejected.

Another method, making use of a partial pressure gauge, will overcome the problem of determining the hydrogen pressure

against the background pressure in that the pressure of each species can be measured with this instrument. It will be necessary, however, to bake-out the manifold so that the systems can be sealed off from the pumps and the pressure still not rise above 10^{-4} torr.

Charge Carriers and Solvation

The charge carrier change with solvation was found to follow, in a rough way, what had been predicted by earlier workers in the field; that is, the amount of protonic conductivity increased with increasing amounts of adsorbed water. However, the general thought was directed along the line that it was not until two to three BET monolayers had formed that protonic conduction would become appreciable.

On the basis of the present study, the question of the onset of protonic conductivity is not quite settled for all of the compounds which were tested. For the samples of melanin, lecithin, and collagen, the current was of a sufficiently high level that it was possible to extend the electrolysis measurements to the one BET monolayer hydration level. If these materials were to be called "pure electronic conductors" in the dry state, the transition from mixed to electronic conduction would have had to occur in a very small hydration range. There does not seem to be, at this time, any theoretical justification for believing that less than one BET monolayer could alter the conduction properties to such a degree. Indeed, in the case of collagen, the material

has a negative slope of percent protonic conduction vs. hydration, and thus when dry would be a protonic conductor. (Collagen, though, is never completely dry; high temperatures used for a through drying produce a change in the material as evidenced by a different adsorption isotherm.) For these experiments, collagen would contain some residual water, as is true for all systems, in the folds of the triple helix which could contribute to its observed protonic conductivity in the "dry" state.

Only in the cases of DNA + water and hemoglobin + methanol was there an experimentally verifiable large change over a small solvation range (in units of BET monolayers). This was a rapid change from electronic carriers to protonic ones.

The basic premise of the electrolysis experiments is that protonic conductivity must result in the evolution of hydrogen. This is a consequence of the fact that metals are "blocking electrodes" with respect to protonic flow. The hydrogen evolution would not have occurred if another type of electrode, potassium chloride in agar, for example, had been used. Indeed, this latter type is "blocking" for electrons.

It is believed that the evolution of hydrogen reflects the protonic flow, and that this flow is the only ionic species. Protons are the only ions possessing a sufficiently high mobility, and being present in sufficient concentration, to be responsible for the currents observed.

It is found for hydrated proteinsthat the current as a function of hydration can be given by Equation 17. It has now been found that the current is a function of two currents, viz., the protonic and the electronic. It is possible to calculate the respective protonic and electronic currents as a function of hydration. This is shown for the case of cytochrome-c (which even shows a crossover point at 2½ BET monolayers) in Figure 42. As can be seen, both the electronic and the ionic components follow Equation 17. That is to say

$$\sigma^{(+)} = \sigma_{dry}^{(+)} \exp (\beta m) \qquad (41)$$

and

$$\sigma^{(-)} = \sigma_{dry}^{(-)} \exp (\gamma m) \qquad (42)$$

where β and γ are constants, and m is the amount of adsorbed water, and $\sigma^{(+)}$ represents the protonic current and $\sigma^{(-)}$ represents the electronic current. The total current is then the sum of these two, or

$$\sigma = \sigma^{(+)} + \sigma^{(-)} = \sigma_{dry}^{(+)} \exp (\beta m) + \sigma_{dry}^{(-)} \exp(\gamma m) \qquad (43)$$

These equations are applicable in the region from zero to about 2½ BET monolayers, that is, before current saturation occurs.

It has been shown (Postow, 1968) for the systems of hemoglobin (with water, methanol, and ethanol), melanin (with water) and collagen (with water) that the activation

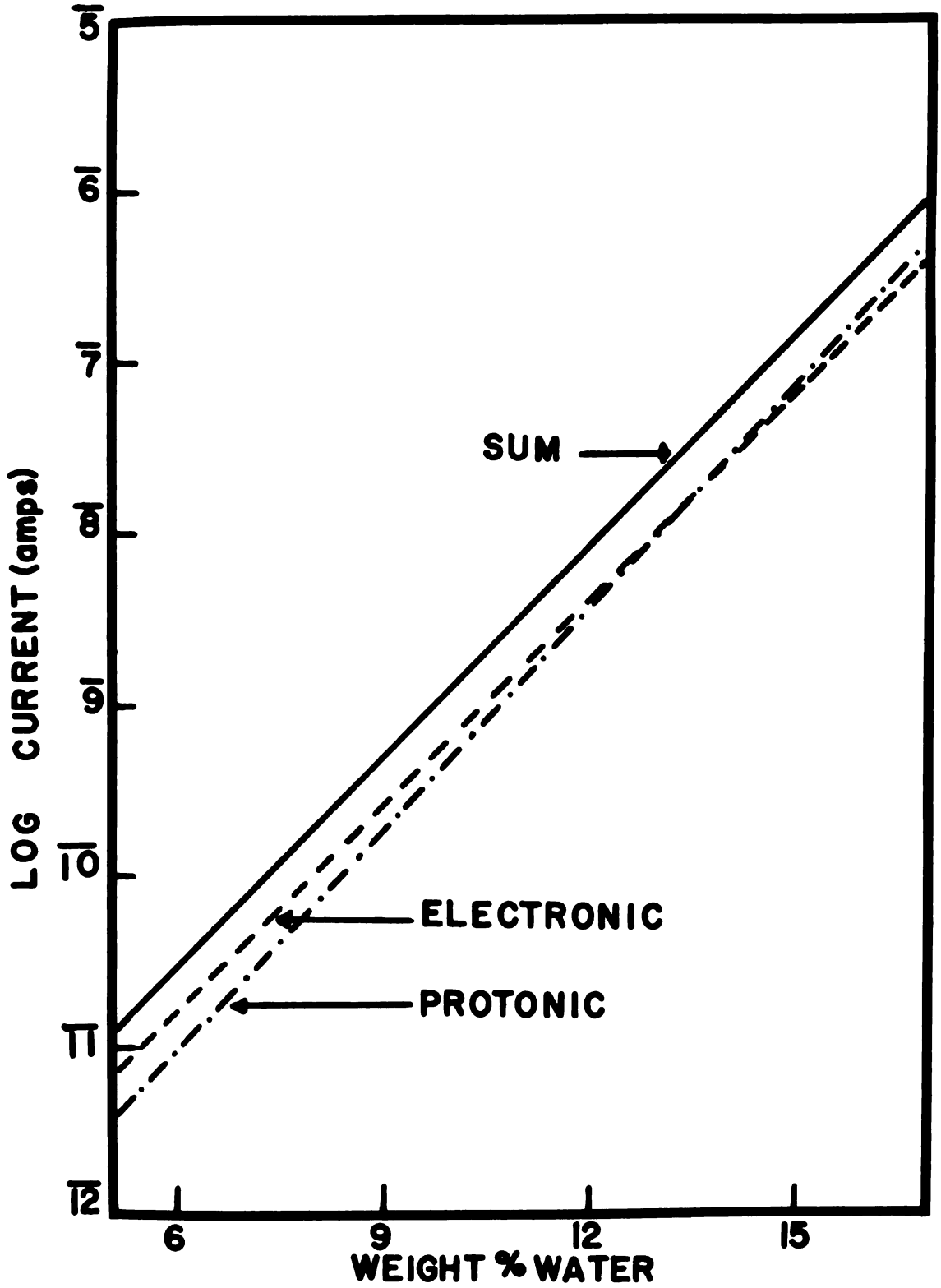


Figure 42. The resolution of the current increase upon hydration into ionic and electronic components.

energy changes upon solvation, and that the value of σ_o in Equation 16 is a constant. This indicates that charge carriers are not added in the solvation process, and that it is only the value of E which changes.

It can also be seen for the case of melanin, that the ratio of charge carriers is a constant for the range of from one to three BET monolayers. From unpublished work of Postow, it is known that the activation energy changes upon hydration in a manner similar to all other materials tested. Thus, one should not associate the change of activation energy, then, with a shift from one carrier to another with each possessing a different value of E. The energy needed to overcome the coulombic attraction of the charge carriers and their "parent" molecule appears to be the principal energy barrier and is the same for protons and electrons.

One can then take Equations 41 and 42 and from them proceed in a manner similar to Equation 17.

$$\sigma_{(T,m)}^{(+)} = \sigma_o^{(+)} \exp\left[(-E_D/2kT) + \beta m\right] \quad (44)$$

and

$$\sigma_{(T,m)}^{(-)} = \sigma_o^{(-)} \exp\left[(-E_D/2kT) + \gamma m\right] \quad (45)$$

so that one gets for each species of charge carrier

$$\sigma_{(T,k')}^{(+)} = \sigma_o^{(+)} \exp\,(-E_D/2kT) \exp\left[(e^2/2kTR_1)(\tfrac{1}{\kappa} - \tfrac{1}{\kappa'})\right] \quad (46)$$

and mutatis mutandi for the electronic carriers.

The total current is then given by the equation

$$\sigma(T,\kappa') = \left[\sigma_o^{(+)} \exp\left\{(e^2/2kTR_1)(\tfrac{1}{\kappa} - \tfrac{1}{\kappa'})\right\} + \sigma_o^{(-)} \exp\left\{(e^2/2kTR_2)(\tfrac{1}{\kappa} + \tfrac{1}{\kappa'})\right\}\right] \exp(-E_D/2kT) \quad (47)$$

If the values of the radii of polarization R_i are about the same, the equation will reduce to

$$\sigma(T,\kappa') = (\sigma_o^{(+)} + \sigma_o^{(-)}) \exp(-E_D/2kT) \exp\left\{(e^2/2kTR)(\tfrac{1}{\kappa} - \tfrac{1}{\kappa'})\right\} \quad (48)$$

and is the form usually found.

In the case of some materials, such as hemoglobin + methanol and DNA + water, the current does not exhibit a saturation with large amounts of adsorbate (about two BET monolayers). It can be seen from Figure 43 that saturation usually occurs within one order of magnitude from a deviation from the linear region in a log current vs. hydration plot; the end of the linear region is indicated by the dotted line. For the DNA and methanol-hemoglobin systems, the change of charge carrier species is not linear with increasing solvation, but instead it seems to show a large change at the point where saturation would normally occur. No doubt, some other effect is taking place; this may be a conformation change as is most likely the case with DNA. This is the "disorder to A-form" transition which occurs at 75% relative humidity (Franklin and Gosling, 1953; Falk et al., 1963a, 1963 b).

The saturation of current which occurs from two to three BET monolayers is the result of the rapid change in the bulk dielectric constant of the material which makes

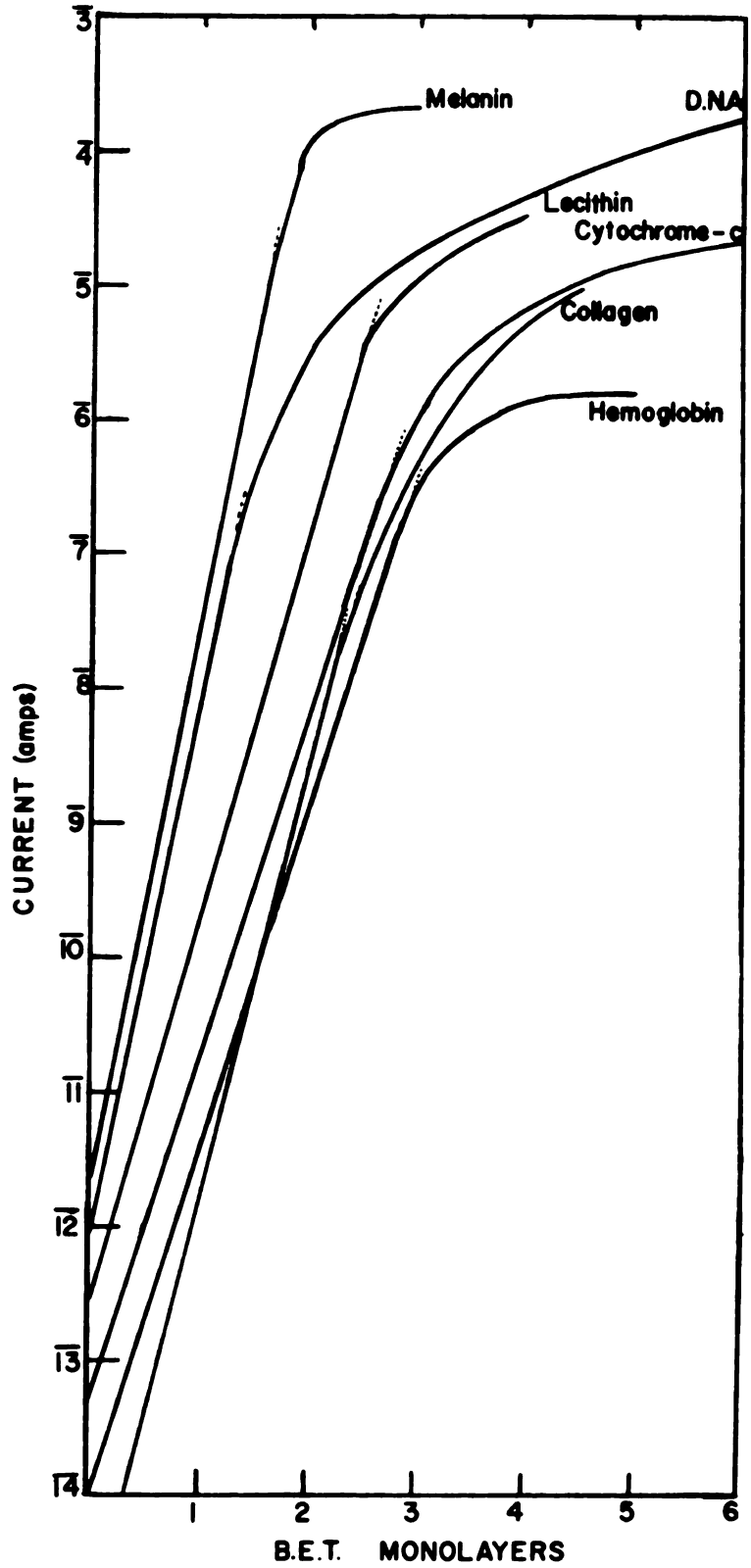


Figure 43. The conductivity of the biomacromolecules used in this study as a function of adsorbed water(in BET).

$1/K'$ very small and reduces the exponent in Equation 26 to a constant. Figures 31, 32 and 33 show this increase in capacitance for hemoglobin, cytochrome-c, and lecithin, respectively.

The adsorbed water is most likely quite polarized with regard to its orientation on the macromolecule in that the adsorption process is found to obey the Bradley isotherm. That is to say, the theory is based on the polarization of one adsorbed layer by the substrate beneath it. This theory has been criticized by Brunauer et al. (1938),but they do state that "...if the adsorbed gas has a large permanent dipole, it is possible that many layers may be successively polarized..." It may be that after two to three BET monolayers, the rotation of the molecule may increase to allow the large increase in the bulk dielectric constant which is observed.

The difference in the observed ratios of the electronic to protonic charge carriers in the different materials tested illustrates the large diversity of types in the conduction process. The range was one of from pure protonic (collagen) to an almost even mixture (melanin) to pure electronic (DNA).

In the materials tested with tritiated water (collagen and hemoglobin), the yield of tritium was small. This could possibly be the result of a different process for the release of hydrogen in the solid-liquid system as compared with the liquid (calibration) system, or it could reflect

the fact that the hydrogen was evolved only from the protons of the adsorbent. Since the mole per cent of tritium in the labeled water was small, it would be possible to label only a small mole per cent of the protons of the protein. The exchanged protons would also be required to be current carrying protons if one were to expect the same yield of tritium/coulomb as from the liquid water used for the calibration; all protons in water are potentially "current carrying protons".

The change in per cent yield of hydrogen at a constant state of hydration also gives some indication that the electrolysis is not the simple decomposition of water found in liquid systems. This change, shown in Figure 41, occurs at about fifty volts, the hydrogen yield falling off below this voltage. If "simple electrolysis" was occurring, the change should come at the decomposition potential of water, 1.35 volts.

In the voltage-resistance plots, it was seen that the resistance showed large changes at potentials of less than thirty volts applied (field strength = 300 v. $cm.^{-1}$). This is also at a higher potential than the decomposition voltage of water.

In an earlier study by Powell (1966), it was found that the current increasing capability of adsorbates was a function of the dielectric constant of the adsorbate. Thus a compound such as dimethyl sulfoxide (DMSO) which has no ionizable hydrogens, but which has a large dielectric

constant, 49, could effect a large current increase when adsorbed on hemoglobin. If the protonic current is significant, as has been found for hemoglobin / methanol, then these protons must assuredly come from the polymer itself. It is obvious, however, from the data on the hemoglobin / methanol system, that not all adsorbates are equally effective in promoting protonic conduction if it is intrin ic to the adsorbent. But protonic conduction, when it does occur for the before mentioned system and for the DNA / water system, sets in sharply, and there is no evidence of current saturation with increasing solvation.

It was hoped that some information concerning the nature of the charge carriers could be obtained from the resistance-voltage plots thus allowing an inference to be made about the other materials whose high resistances prevented electrolysis experiments. This has however seemed to be a futile endeavor. The plots are more characteristic of the material than of the charge carriers. Except for high solvation states in the methanol / hemoglobin system, the plots are similar to the water / hemoglobin system. Thus little can appear to be learned from the resistance-voltage plots of the ethanol / hemoglobin system, Figure 30. There even seem to be differences between cytochrome-c, Figure 24, and hemoglobin, Figure 28, yet their pattern and degree of charge carrier changes are quite similar.

Last of all, one should note that the difference between the kinetics of the current increase from dryness

and the weight increase from dryness as a function of time (illustrated in Figure 14 for one case) indicates that the current increase is mainly effected by conduction in the more superficial layers of the crystallites, a previously suspected idea.

It must be pointed up, of course, that many questions still remain, and the case has not been proved that protonic conduction is intrinsic. Several methods by which this could be demonstrated are possible. One such method would involve the resolution of the activation energy plots into the electronic and protonic components and then shown that there indeed exists a σ_o^+ and a σ_o^-. This type of calculation has heretofore been impossible to do because of the amount of variation in the slopes of the lines in the activation energy plots leading to poor values of the y-intercept. This is an experimental difficulty and is not easily resolvable; large extrapolations of the data are involved and small errors become greatly magnified.

If attempted, the proceedure would simply involve hydrating a sample to a known value of p/p_o and then finding its amount of regain from the adsorption isotherm and the amount of protonic and electronic conduction from the graphs in this thesis. The small errors in the activation energy plots are most likely the result of variations in the amount of adsorbed water during the heating cycle. This evaporation of the water could partially be reduced by increasing the pressure of the ambient (non-

adsorbed) gas surrounding the sample. One could pressurize the chamber to two atmospheres of nitrogen, for example.

This would answer the question of the "intrinsic" nature of the protonic charge carriers but would not answer the question if the protons were being replaced by the adsorbed vapor. This would be required for the case where currents of a relatively large magnitude are passed for long periods of time (milliamps for a few minutes) because "intrinsic protons", those present in the biomacromolecule, are not present in sufficient quantity for sustained currents. By making use of a partial pressure gauge, and an isotope label in the adsorbate (or adsorbent), this question could be resolved by an examination of the electrolysis products. This work is in progress at the time of this writing.

Another unanswered question is the nature of the charge carrier at low applied potentials. That unusual effects are occurring (with respect to protonic conduction) is evidenced by the non-ohmic behavior below 30 volts applied and the low hydrogen evolution as measured in the electrolysis apparatus. The high over-voltage for hydrogen production could result in the current carriers being either electrons or some other ion, as yet unidentified. Ionic carriers, however, present theoretical problems because of their low concentration and their supposedly low mobility. Again, the partial pressure gauge could prove useful if a gaseous product was involved.

Interesting questions arise with respect to the temperature dependence of the charge carrier species. Does the amount of protonic conduction increase in the biomacromolecules with temperature as was found for nylon-66? Higher temperatures would also increase the conductance and would thus allow measurements to be made at lower solvation ranges. If the activation energy for the production of protons and electrons is equal (or approximately so), as is suggested here, there should not be found any change in the electronic/protonic charge carrier ratio with temperature, only with solvation. This could be an important point for poikilotherms if electronic processes are to be important in biological systems.

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